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Development, production and characterisation of the proposed Second WHO International Standard for Antibodies to Varicella Zoster Virus

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NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposal(s) contained therein. Written comments on the proposal(s) MUST be received in English by **5 October 2026** and should be addressed to:

Biologicals Norms and Standards and Transplantation
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The distribution of this document is intended to provide information to a broad audience of potential stakeholders and to improve the transparency of the consultation process. Following consideration of all comments received, the proposal(s) will then be considered by the WHO Expert Committee on Biological Standardization (ECBS) prior to a final decision being made and published in the WHO Technical Report Series.

Summary

The 1st International Standard for Varicella Zoster Immunoglobulin, coded W1044, was established by WHO in 1987 and has been widely used for the calibration of assays intended to measure anti-varicella zoster virus (VZV) antibody levels in purified immunoglobulin products and clinical samples. Due to depletion of stocks of W1044, two candidate replacement standards have been produced by MHRA for evaluation. The first candidate standard, coded 25/136, is a lyophilised preparation of normal human plasma. The second candidate standard, coded 25/168, is a lyophilised preparation of purified human VZV immunoglobulin.

Based on the results obtained in an international collaborative study involving 15 laboratories from 7 countries both candidate standards are considered to be fit for purpose as a replacement for W1044. There are no differences in terms of batch homogeneity or stability profile and both candidate standards show good commutability across the different sample types included in the study. The “like for like” purified immunoglobulin candidate preparation 25/168 showed better parallelism with W1044 than did 25/136, and better continuity of IU (in terms of the potential shift that would be observed for potency estimates for immunoglobulin samples). For that reason, candidate preparation 25/168 is recommended as the replacement for W1044, for adoption as the 2nd WHO International Standard for Antibodies to Varicella Zoster Virus with a proposed assigned value of 16 IU/ampoule.

1. Introduction

An International Standard for Varicella Zoster Immunoglobulin was first established by WHO in 1987 (product coded W1044) [1,2]. The standard was intended to be used for the calibration of immunoassays (and secondary standards) that are used to measure anti-varicella zoster virus (VZV) antibody levels with results reported in International Units (IU). These immunoassays are used with clinical samples (human serum or plasma) to assess immunity or to assess the response to vaccination with VZV vaccines. They are also used to measure potency of purified immunoglobulin preparations used in immunosuppressed patients to control infection [3].

Since its establishment, the 1st IS has been used by blood product manufacturers, vaccine manufacturers, clinical diagnostics manufacturers and public health, academic and regulatory laboratories. Stocks of the 1st IS are now close to exhaustion and a proposal from MHRA to develop a replacement IS was endorsed by the WHO Expert Committee on Biological Standardisation at its 77th meeting in March 2023 [4]. Two candidate replacement standards were prepared, one using a purified immunoglobulin product kindly donated by a blood product manufacturer and the other using plasma kindly donated by a plasma collection company. Because the current standard is used to standardise assays that measure anti-VZV levels in purified immunoglobulin and clinical samples, two candidate standards were produced to determine which type of standard would perform best across both sample types. The two candidate standards were evaluated in an international collaborative study involving 15 laboratories in seven countries, representing two WHO regions. The aim of the collaborative study was to assess performance of the candidate standards in commonly used immunoassays and to estimate a potency in IU relative to the 1st IS. A small number of purified immunoglobulin samples from different commercial products were included in the study for an assessment of continuity, and a larger number of clinical samples (plasma and serum) were included to assess commutability of the candidate standards. We report here the results of this multi-lab evaluation, together with the results from stability and homogeneity assessments conducted by MHRA.

2. Bulk material

The source material used to prepare the plasma candidate standard consisted of a pool of three individual plasma donations (from immunised donors) collected in the USA, kindly donated by a plasma collection company. The source material used to prepare the immunoglobulin candidate standard consisted of 5% purified hyperimmune VZV immunoglobulin derived from VZV-immunised donors and was kindly donated by a blood products manufacturer. Both source materials were tested and shown to be negative for antibodies to HIV, HBsAg and HCV RNA.

3. Processing of the candidate WHO International Biological Reference Standards

The bulk material for each candidate standard was filtered prior to filling into 2.5 mL DIN type I glass ampoules with a target fill weight of 0.5 g per ampoule. Filling was performed at the MHRA's Centre for Biological Reference Materials on an AVF5090 filling line (Bausch & Stroebel, Ilshofen, Germany) in June 2025 for the plasma source material and September 2025 for the immunoglobulin source material with on-line check-weighing of a proportion of the filled ampoules. Filled ampoules were partially stoppered with halobutyl 13mm diameter igloo closures and lyophilised in a CS100 freezer drier (Serail, Le Coudray Saint Germer, France). Freezing was performed to -50˚C over 2 hours then held for a further 2 hours before applying vacuum. Primary drying was performed at -35˚C for 40 hours at 100 µbar vacuum followed by a ramp to 25˚C over 10 hours, then 30 hours secondary drying at 25°C and 30 µbar vacuum. Ampoules were back filled with dry nitrogen to atmospheric pressure stoppered in the dryer before removal and flame sealed. The sealed ampoules were then stored at -20°C under continuous temperature monitoring. A total of 4033 ampoules were filled for the plasma candidate.

A total of 4517 ampoules were filled for the immunoglobulin candidate. The candidate standards were coded 25/136 for the plasma standard and 25/168 for the immunoglobulin standard. Both were stored at -20°C.

3.1 Quality control testing of the candidate standards

The freeze-dried products, 25/136 and 25/168, were successfully lyophilised forming a robust, off-white cake. Fill precision was assessed by weighing ampoules that were sampled throughout the filling run. For 25/136 a total of 144 ampoules were weighed, with a mean fill mass of 0.51 g and a coefficient of variation of 0.81%. For 25/168 a total of 315 ampoules were weighed, with a mean fill mass of 0.51 g and a coefficient of variation of 0.56%. Equilibrium Relative Humidity (ERH) (%) and oxygen content (%) were measured after sealing as indicators of successful freeze-drying and ampoule integrity respectively. Equilibrium Relative Humidity was measured non-invasively using frequency modulated spectroscopy water activity analyser.

The mean ERH% was measured in 12 ampoules of 25/136 was 1.29%. The mean ERH% in 12 ampoules of 25/168 was 1.24%. Oxygen content was measured non-invasively by frequency modulated infra-red spectroscopy using an FMS-760 Oxygen Headspace Analyzer (Lighthouse Instruments, Charlottesville, VA, USA). The mean oxygen headspace measured in 12 ampoules of 25/136 was 0.31% and for 25/168 it was 0.25%. Microbial bioburden testing for bacterial and mould/yeast contamination were performed on pre-fill, post-fill and lyophilised samples and revealed a low-level of bioburden (<10 cfu/mL) for all tested samples. A full summary of the quality control test results is shown in Table 1.

4. Stability studies

An accelerated thermal degradation study was conducted to predict the long-term stability of both candidate standards and to inform decisions related to shipment of the product at ambient temperature. Ampoules of the candidate standards were stored at +4°C, +20°C, +37°C and +45°C, in addition to the normal storage temperature of -20°C. Ampoules were retrieved at defined intervals up to 12 months (Tables 2, 3) and then assayed for anti-VZV antibodies using the DiaSorin LIAISON® VZV IgG HT assay. Estimates for each sample, relative to the -20°C sample, were calculated by sigmoid curve analysis using European Directorate for the Quality of Medicines & Healthcare (EDQM) software CombiStats™ v1.2.2 [5] and are shown in Table 2 (for 25/136) and Table 3 for (25/168). The data did not fit the Arrhenius model [6] for accelerated degradation studies and no prediction of long-term stability could be made. At the last time point (12 months for 25/136 and 11 months for 25/168) the potency of the ampoules held at -20°C was calculated relative to ampoules held at the long-term baseline of -70°C to obtain an estimate for real-time stability. This gave estimates of 1.09 (95% confidence limits 1.05 – 1.12) for 25/136 and 1.03 (95% confidence limits 0.98 – 1.07) for 25/168 suggesting that both candidate standards are stable.

5. Assessment of Homogeneity

The precision of fill provides good evidence for the homogeneity of the batch in terms of the amount filled per ampoule. The precision of fill (CV% of the mean filling mass) was 0.81% for 25/136 and 0.39% for 25/168, both of which are acceptable for a plasma sample with a fill volume of 0.5 mL per ampoule.

To provide further assurance regarding homogeneity of the batch, anti-VZV antibody levels were measured in 10 different ampoules using the DiaSorin LIAISON® VZV IgG HT assay. One ampoule was taken from the beginning (Ampoule 1), one ampoule from the middle (Ampoule 2) and one ampoule from the end (Ampoule 3) of the fill and 7 other ampoules were selected at random across the fill. For each candidate standard, the set of 10 ampoules was tested in three independent assays. Potency estimates for all ampoules were calculated relative to Ampoule 1 using CombiStats™. Geometric mean potency estimates combined across assays for each vial are summarised in Table 4. For both 25/136 and 25/168, these results gave negative estimates of the uncertainty of measurement component for ampoule heterogeneity, indicating that no heterogeneity could be detected in these assays, providing good evidence for homogeneity of the batch

6. Collaborative study

6.1 Participants

Laboratories were invited to participate based on their expertise and experience in performing immunoassays measuring anti-VZV antibodies, and to ensure that a range of assays currently used in the field were represented in the study. Nineteen laboratories agreed to participate in the study, however three laboratories were unable to contribute data for this report due to

significant delays obtaining import permits for the study samples, and one laboratory withdrew due to insufficient resources to complete the testing. The 15 laboratories who did receive samples and returned data to MHRA are listed in Appendix 1. These laboratories were from 7 countries, representing two WHO regions: Germany (6), Belgium (1), Czech Republic (1), France (1), Italy (1), Japan (1) and UK (4). Participating laboratories were from commercial diagnostics manufacturers, therapeutic product manufacturers, public health and national control laboratories. All participating laboratories are referred to in this report by a code number randomly allocated and not related to the order of listing in Appendix 1.

6.2 Collaborative Study Samples

Participants received the 1st IS W1044 and the lyophilised candidate 25/168 (study panel code VZV-02) as well as the liquid bulk of 25/168 as a frozen aliquot (VZV-01). They also received the lyophilised candidate 25/136 twice as coded duplicates (VZV-07 and VZV-08) and a frozen aliquot of the liquid bulk of 25/136 (VZV-06). Additionally, participants received (as frozen aliquots) 3 samples of different commercial purified immunoglobulin products from 3 different manufacturers (VZV-03 to VZV-05), 14 human plasma samples (VZV-09 to VZV-22), 9 human serum samples (VZV-23 to VZV-31) and an IgG/ IgA depleted serum (VZV-32). 22 of the human serum and plasma samples were from routine blood donations from adult donors and were sourced from the UK NHS Blood and Transplant service (NHSBT). One of the plasma samples was collected from a donor in the USA and kindly donated by a plasma collection company. All samples were tested at MHRA to confirm negativity for antibodies to HIV, HBsAg and HCV RNA and were pre-screened at MHRA using the DiaSorin LIAISON® VZV IgG HT assay to ensure that the study panel contained samples with a range of anti-VZV antibody levels. The human plasma and serum samples were not pooled (i.e. they donations from a single individual). All plasma and serum samples were filtered through Nalgene 0.2µM filters prior to aliquoting and distribution to study participants.

The full list of study samples showing the traceability for the identifiers used in this report is shown in Table 5.

6.3 Study Design

The study protocol is provided in Appendix 2 and was conducted under MHRA study code CS744. For each method contributed by a participating laboratory, participants were asked to perform three independent runs, using a fresh set of samples for each test. Participants were provided with an extra set of samples to allow for a preliminary assay where desired.

Participants were asked to test the 1st IS (W1044), the candidate replacement standards (25/136 and 25/168) and the purified immunoglobulin samples (VZV-03 to VZV-05) using serial dilutions to reach beyond the assay end point. Participants were asked to test the plasma (VZV-09 to VZV-22), serum (VZV-23 to VZV-31) and immunoglobulin depleted (VZV-32) samples according to their routine practice for testing clinical samples. For quantitative methods, participants were asked to repeat the test for clinical samples where the potency was above the assay quantitative range, using a higher starting dilution for the sample.

A results-reporting sheet was provided for participants to record all essential information including the raw data from each run of each assay method they performed.

6.4 Assay Methods

Assays used by the participants are shown in Table 6. The assay methods used in the study were detecting IgG binding antibodies against virus antigen, and most participants used a commercial assay (manual immunoassay kit or automated analyser) with two laboratories performing an in-house assay. The assays were based on either absorbance or chemiluminescence detection. Where laboratories performed multiple assay methods, laboratory codes are followed by a letter indicating the different methods (e.g. Lab 2a, 2b).

6.5 Collaborative Study Results – Value assignment and continuity

6.5.1 Statistical analysis

Potencies of samples VZV-01 to VZV-08 were calculated in milli-International Units (mIU)/mL relative to the current IS, W1044. For all assays, parallel line analysis was performed, using a linear section of the response range with a minimum of three dilutions for all samples (where provided). Calculations were performed using R [7]. Non-linearity and non-parallelism were considered in the assessment of assay validity. Instances where the coefficient of determination R^2 value was >0.98 were considered to show acceptable linearity, and parallelism was concluded to be acceptable where the ratio of the fitted slopes for the test and reference samples was within the range 0.80-1.25. Cases not meeting these criteria were considered invalid and no estimates are reported.

Results from all valid assays were combined to generate unweighted geometric mean (GM) estimates for each laboratory and these laboratory means were used to calculate overall unweighted geometric means for each sample. Variability between assays and between laboratories has been expressed using geometric coefficients of variation ($GCV=\{10^s-1\} \times 100\%$, where "s" is the standard deviation of the $\log_{10}$ transformed estimates). Due to possible outliers and anomalous results, overall median estimates and Huber's robust GM estimates were also calculated using the R package 'WRS2' [8]. Inter-laboratory variability was also quantified as interquartile ranges (IQR), expressed as the fold-change UQ/LQ, where UQ and LQ denote the upper and lower quartiles respectively. Calculations for median, LQ and UQ were performed on a log scale before being back-transformed to the original scale of measurement.

6.5.2 Data received from study participants and assay validity

Results were received from 15 laboratories, comprising a total of 18 data sets. Where laboratories performed more than one method the results were treated as if from separate laboratories and coded (for example, laboratories 02a and 02b). Laboratory 12a was excluded from the analysis as samples were tested at a single dilution only, and laboratory 12b was excluded from the analysis as qualitative data was reported, therefore parallel line analysis

could not be performed in either case. Laboratory 14 was also excluded from the analysis as they did not receive the current or candidate IS samples.

The majority of assays performed gave valid estimates of potency for all samples relative to the current IS W1044. Slope ratios for these samples relative to the current IS, used as a measure of parallelism, ranged from 0.70 to 1.49, with 93% within the range 0.80-1.25, and are shown in Supplementary Figure 1. A trend for slope ratios to be closer to 1.0 was noted for samples VZV-01 and VZV-02, indicating that 25/168 showed more consistent parallelism with the current IS, W1044, compared to 25/136. The immunoglobulin samples (VZV-03 to VZV-05) showed better parallelism with candidate IS 25/136 (VZV-07/VZV-08) and more variable slope ratios across assays and laboratories relative to W1044 as shown in Supplementary Figure 1. The minimum R^2 value for all samples was 0.92, with 90% of values >0.98, as shown in Supplementary Figure 2.

6.5.3 Intra-laboratory variability

Intra-assay variability was assessed using assays where a valid estimate was obtained for both of the coded duplicate samples VZV-07 and VZV-08. Estimates of VZV-07 expressed as a percentage of the estimate for VZV-08 in the same assay were in the range 82-121% in all cases, and within 90-111% in 94% of cases, illustrating an acceptable low level of intra-assay variability in the results from this study.

For each laboratory, where two or more valid estimates of relative potency were obtained for samples VZV01 to VZV-08, inter-assay variability was expressed as the ratio of maximum to minimum estimate obtained for the sample. An acceptable level of inter-assay variability was evident from the calculated values with this ratio being <2 in 97% of cases (n=100).

6.5.4 Potency estimates of candidate standards 25/168 and 25/136

Potency estimates relative to the current IS in mIU/mL are summarised in Table 7a and Figure 1. Samples VZV-07 and VZV-08 (the coded duplicates of 25/136) were also combined to give an overall potency estimate for this candidate preparation. Individual assay relative potency estimates are shown in Supplementary Table 1.

Estimates for 25/168 (VZV-02) ranged from 19715 mIU/mL (lab 17) to 42938 mIU/mL (lab 10) with an overall GM of 31315 mIU/ml, an inter-laboratory GCV of 24.02%, and an IQR of 1.15. The distribution of log transformed estimates showed no significant deviations from normality and no laboratories were identified as significant outliers. Calculation of overall estimates as median and robust GM gave a value of 32150 mIU/mL and 32201 mIU/mL respectively. The results obtained therefore support assignment of 32000 mIU/mL (16000 mIU/ampoule) to this candidate standard.

Estimates for 25/136 (VZV-07/VZV-08) ranged from 1848 mIU/mL (lab 04) to 6692 mIU/mL (lab 05b) with an overall GM of 4064 mIU/mL. 25/136 showed higher variability than 25/168,

with an inter-laboratory GCV of 40.34% and an IQR of 1.49. Calculation of overall estimates as median and robust GM gave a value of 4075 mIU/mL and 4163 mIU/mL respectively. Given the high GCV these results would support a final estimate of 4000 mIU/mL (2000 mIU/ampoule).

Twelve laboratories reported results in mIU/mL and a summary of these results is shown in Table 8. For six of these laboratories, the geometric mean estimate of W1044 is within 80-125% of the assigned value of 50000 mIU/mL for this International Standard. For the remaining laboratories, estimates were 74% higher (lab 03), 107% higher (lab 04), 91% lower (lab 05b), 45% higher (lab 10), and 108% higher (lab 15).

6.5.5 Potency estimates of immunoglobulin samples VZV-03 to VZV-05

Potency estimates of immunoglobulin samples VZV-03 to VZV-05 calculated relative to the current IS in mIU/mL are summarised in Table 7b. Samples showed a similar level of inter-laboratory variability, with GCVs of 71.06%, 46.82% and 52.90%, and IQR of 1.45, 1.69 and 1.83.

The estimated potencies of the immunoglobulin samples were also expressed relative to the candidate standards 25/168 and 25/136, assuming assigned values of 32000 mIU/mL and 4000 mIU/mL respectively. These results are summarised in Tables 9 and 10. Similar inter-lab GCVs and IQRs were observed for all of the immunoglobulin samples when expressed relative to the candidate 25/168 compared to the current IS. For estimates relative to the candidate 25/136, there was an improvement in inter-lab GCV and IQR compared to the current IS, for all of the immunoglobulin samples. Tables 9 and 10 also show the immunoglobulin sample estimates relative to the candidate IS as a percentage of the potencies calculated relative to the current IS. For the samples relative to 25/168, these percentages range from 74.5% to 162.3%, with the majority between 90% and 111%. For the samples relative to 25/136, a slightly wider range of percentages was observed, with the range from 59.8% to 216.4%. This illustrates the range of possible shifts that may be observed when changing from the current to replacement IS and suggests better continuity of estimates for samples of this type with 25/168 compared to 25/136.

The overall geometric mean potency estimates for samples VZV-03 to VZV-05 relative to the candidate standards were also expressed as a percentage of the laboratory reported estimates where those were reported in mIU, as shown in Tables 11 and 12. Most of the percentages are within 70% and 143% for both candidate standards.

6.6 Collaborative Study Results – Commutability assessment

6.6.1 Statistical analysis

Commutability of the current IS, W1044, the candidate IS, 25/168, and the candidate IS 25/136, with 3 immunoglobulin samples, 14 plasma samples and 10 serum samples, was assessed by a "calibration effectiveness" approach [9] using data from quantitative assays calibrated against

the current IS W1044. Only laboratories that tested all 27 samples, the current IS and the two candidate standards were included in this analysis (10 laboratories in total). Serum sample VZV-32 was excluded from the analysis as it was (correctly) reported as negative by the majority of laboratories. Laboratories 01, 06 and 16 were excluded from this analysis as they did not test the plasma and serum samples. Laboratory 05a did not test 25/136 so to allow consistent comparison for both candidate preparations their data were excluded from this analysis. The assay performed by laboratory 19 gave inconsistent results across different runs for the plasma and serum samples, so they were also excluded from the commutability assessment.

For the immunoglobulin samples, estimates previously calculated relative to the three standards (Tables 7b, 9 and 10) were used. For the plasma and serum samples, estimates were calculated by parallel line analysis as in the value assignment part of the study where possible, but in the majority of cases by interpolation from the fitted dose-response curve for W1044 as the samples were only tested at single dilutions by most laboratories. Estimates relative to 25/168 or 25/136 were then calculated using the ratios of a sample's value to 25/168 or 25/136 respectively, scaled according to the proposed values for the relevant reference being used (section 6.5.4).

Laboratory geometric mean estimates expressed as a % of study median estimate for the sample (used as target value for the purpose of this analysis) were used to assess the extent to which laboratories agreed with consensus estimates. A value of 100% indicates complete agreement with the sample target value, so the relative activity of test sample and standard is consistent with other labs and therefore the standard is commutable in such cases. In order to derive an acceptable range (for analysis of this study only) for concluding commutability, the standard deviation of log transformed values for immunoglobulin samples was calculated using results relative to W1044 within each laboratory, and a pooled value, s_P, was calculated across all laboratories. The acceptable range was then set as $\pm 3 s_P$. For this study this gave a range of 0.553-1.809. This means that the values should be demonstrated to be within the range 55.3% to 180.9% for acceptable commutability to be concluded.

6.6.2 Commutability results

Laboratory geometric mean estimates for the immunoglobulin, plasma and serum samples calculated relative to W1044, 25/168 and 25/136 are shown in Tables 13, 14 and 15, assuming potencies of 50000 mIU/mL, 32000 mIU/mL and 4000 mIU/mL for these samples respectively when used as the standard. Overall study values for each sample are shown as GM and median estimates (calculated using log transformed data), with inter-laboratory variation expressed as both GCV and Inter-Quartile Range (IQR) values.

The laboratory geometric mean sample estimates expressed as a % of study median estimate for the sample are shown in Tables 13a (estimates relative to W1044), 14a (estimates relative to 25/168) and 15a (estimates relative to 25/136) and values which fall outside of the

commutability acceptance range for this study are shaded. This data is also shown in Figure 2 (estimates relative to W1044), Figure 3 (estimates relative to 25/168) and Figure 4 (estimates relative to 25/136) where the commutability acceptance range for this study (55.3% – 180.9%) is indicated by the dashed horizontal lines. The summary calculations per laboratory are shown in Tables 13b (estimates relative to W1044), 14b (estimates relative to 25/168) and 15b (estimates relative to 25/136).

A similar level of commutability with immunoglobulin samples was observed for both the existing IS, W1044, and the candidate IS, 25/168. Full commutability was observed in 7 laboratories for samples relative to each of W1044 and 25/168 and in both cases 25/30 of the lab geomean estimates were within the commutability acceptance range. Commutability with immunoglobulin samples was improved where estimates were expressed relative to the candidate 25/136 where 29/30 of the lab geomean estimates were within the commutability acceptance range.

Commutability with plasma and serum samples was similar for all 3 standards/candidate standards (W1044, 25/168 and 25/136). Median GCV values were 50% for W1044 and 63% for 25/168 and in both cases 78% of the plasma/serum samples had IQR values below 2. For candidate 25/136 the median GCV value was 49% and 74% of IQR values were below 2. The % of lab geomean estimates within the commutability criteria was 81% (for estimates relative to W1044), 80% (for estimates relative to 25/168) and 85% (for estimates relative to 25/136). Lab 17 had the highest number of out-of-range estimates for the plasma and serum samples and excluding this laboratory brings the % of lab geomean estimates that are within the commutability acceptance range to 86%, 87% and 89% for W1044, 25/168 and 25/136 respectively.

7. Discussion

Both candidate standards were successfully filled into glass ampoules and lyophilised. In addition to the lyophilised candidate standards, participants also received a sample of the pre-lyophilised bulk material for each candidate which allows us to estimate the loss of activity on freeze-drying. Using the robust geometric mean of laboratory reported estimates, the loss of potency on freeze-drying was comparable for both candidate preparations (6% for 25/168 and 4% for 25/136) and consistent with the degree of loss that we typically observe after lyophilisation of immunoglobulin and serum/plasma materials. For both candidate standards, the coefficient of variation of the mean filling weight was <1% and therefore considered to be acceptable for a fill with a target fill weight of 0.5g. In addition, no significant heterogeneity was observed after measuring anti-VZV antibody levels in 10 ampoules that were sampled from across the batch of each product. Results from measurement of relative humidity/moisture and oxygen, plus the results from stability studies suggest that both lyophilised candidate preparations will be stable when stored at the recommended storage temperature.

The collaborative study involved 15 laboratories from 7 countries in 2 WHO regions. Laboratories in other WHO regions were contacted and invited to participate but were unable

to join. The collaborative study for the 1st WHO IS for Varicella Zoster Immunoglobulin (W1044) showed that ELISA was suitable for measurement of anti-VZV IgG [2]. Such assays are now widely used and nearly all participants in our collaborative study used such a method. The 1988 collaborative study for W1044 evaluated performance against other purified immunoglobulin preparations. Since then, the standard has been widely used for calibration of immunoassays that are routinely used to measure anti-VZV IgG levels in human clinical samples. Therefore, in addition to preparation of a "like for like" purified VZV immunoglobulin candidate replacement standard (25/168) we also prepared a candidate standard from a pool of 3 human plasma donations (25/136). In addition to a small number of purified immunoglobulin samples, we also included a large number of human plasma and serum samples in the collaborative study sample panel to allow us to evaluate the commutability of each candidate preparation.

The performance of laboratories (as judged by intra-lab variation and results obtained for coded duplicates) in this study was good, and we did not observe any trends related to a particular commercial assay. The majority of assays performed gave valid estimates of potency for all samples relative to the current IS W1044 and the intra-laboratory variation (within assay and between assay) was low. In the absence of established parallelism criteria for all assays a slope ratio acceptance range of 0.80 – 1.25 was chosen as the parallelism acceptance criterion for this study. In order to assess the impact of this acceptance range on the final estimates obtained, the data were assessed using various other slope ratio acceptance ranges between 0.5 and 2 which did not affect the calculated estimates for both candidate standards.

Eleven laboratories returned results from quantitative assays that reported a result in mIU/mL for W1044. For these labs, the geometric mean estimate was within 80-125% of the assigned value of 50000 mIU/mL in 6/11 cases suggesting that these assays are appropriately calibrated. However, in 5 cases the geometric mean estimate for W1044 ranged from 91% lower to 108% higher than its assigned value but there was no apparent association with any particular commercial assay.

The results obtained for the two candidate standards confirmed the higher potency of 25/168 (purified VZV immunoglobulin preparation) compared to 25/136 (pooled plasma preparation) as seen in pre-study testing at MHRA. For the potency estimates calculated relative to W1044, the inter-laboratory variability was higher for 25/136 (GCV 40.34%) than it was for 25/168 (GCV 24.02%) and a trend for slope ratios to be closer to 1.0 was also noted for 25/168 suggesting more consistent parallelism with the current IS, W1044, compared to 25/136.

The level of agreement between laboratories for potency estimates of the 3 immunoglobulin samples was similar when potencies were expressed relative to W1044 or to candidate 25/168. The inter-lab variation was improved when potencies were expressed relative to candidate 25/136. The reason for the higher variation in potency estimates expressed relative to the two purified immunoglobulin preparations (W1044 and 25/168) is not known but might be related

to greater variation in the reconstitution of the lyophilised immunoglobulin preparations (higher Ig %) compared to the normal plasma preparation.

To understand the possible shift in potency estimates for samples of this type that laboratories may observe following replacement of W1044, we expressed potency estimates calculated relative to each candidate standard as a percentage of the estimate calculated relative to W1044. A wider range was observed for 25/136, and a smaller proportion of labs had estimates within 80-125% of the estimate obtained relative to the current WHO IS. This suggests a better continuity of potency estimates for samples of this type with candidate 25/168 compared to candidate 25/136.

All 3 standards/candidate standards showed good commutability with immunoglobulin samples and plasma and serum samples. When preparing our panel of plasma and serum samples, our aim was to provide samples that cover a wide range of anti-VZV IgG levels and the results obtained from the study confirm that this was achieved because within each of the plasma and serum sample groups, the level of anti-VZV IgG covered at least a 10-fold range. The results for some plasma and serum samples were consistently highly variable between labs in all 3 data sets (i.e. with W1044, 25/168 and 25/136) – for example VZV-14, VZV-12 (plasma) and VZV-29, VZV-17 (serum) which consistently gave the highest IQR values across the 3 data sets. The reasons are unknown but it does not relate to anti-VZV IgG level because these samples were not at either end of the median response across all samples within a sample group. Compared to other laboratories, laboratory 17 had the highest number of out-of-range estimates for plasma and serum samples, tending towards a positive bias relative to the study median. This laboratory used one of only 2 in-house methods and the target antigen in the ELISA is the VZV glycoprotein E (gE). Other methods used by other laboratories in the study may have a broader target for VZV antibodies because of the type and purity of the antigens used (Table 6) which may be a contributing factor to the difference in performance in this study.

Based on all of the results obtained, both candidate standards can be considered to be fit for purpose as a replacement for W1044. There are no differences in terms of batch homogeneity or stability profile and both candidate standards show good commutability across the different sample types included in the study. The “like for like” candidate preparation 25/168 showed better parallelism with W1044 than did 25/136 and better continuity of IU (in terms of the potential shift that would be observed for potency estimates for immunoglobulin samples). For that reason, candidate preparation 25/168 is preferred as the replacement for W1044. The higher potency of this preparation may also provide some practical advantages for some uses. The proposed replacement IS may be suitable for use in VZV antibody assays that were not included in this collaborative study but the proposed assigned value for 25/168 is based on the relative potency of 25/168 compared to W1044 in the assays included in this study which were predominantly IgG binding assays.

8. Proposal

The candidate standard coded **25/168** is recommended for establishment as the 2nd WHO IS for Antibodies to Varicella Zoster Virus, with an assigned value of **16 IU/ampoule**.

Following use of material for product quality control testing and collaborative study evaluation, 4,216 ampoules remain available for distribution.

Product coded 25/168 will be stored at -20°C in the Centre for Biological Reference Materials, National Institute for Biological Standards and Control, Medicines and Healthcare products Regulatory Agency, South Mimms, EN6 3QG, UK.

9. Comments Received from Collaborative Study Participants

Due to the short time between completion of this project and report and the October 2026 ECBS meeting, participants were only given a very short window to return their comments before a final version was submitted to WHO for the public consultation. During this window, minor editorial corrections were received from two participants and these have been incorporated into this version of the report. Any further comments, corrections or issues highlighted by other participants, including during the WHO public consultation window, will be highlighted to the ECBS in October and will be addressed in a revised version of this report where necessary.

10. Acknowledgements

We gratefully acknowledge the important contributions of the collaborative study participants and the anonymous donors of the immunoglobulin, plasma and serum study samples. We extend our thanks to the MHRA manufacturing, inventory and logistics teams for their expert management in the formulation and production of the candidate material, storage and distribution of the materials to study participants.

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12. Tables and Figures

Table 1. Profile of the candidate standards 25/136 and 25/168 and results from quality control tests

Product code	**25/136**	**25/168**
Source material	Pooled human plasma	5% Varicella Zoster Immunoglobulin
Presentation	Lyophilised	Lyophilised
Container type and size	2.5 mL DIN Ampoule	2.5 mL DIN Ampoule
Appearance	Freeze-dried white cake	Freeze-dried white cake
No. ampoules filled	4033	4517
Date of fill	24 Jun 2025	12 Sep 2025
Recommended storage temp	-20 °C or below	-20 °C or below
Mean fill mass	0.51 g (CV= 0.81%, n=144)	0.51 g (CV=0.39%, n=315)
Mean equilibrium relative humidity	1.29% (CV=5.82% , n=12)	1.24% (CV=4.47%, n=12)
Mean of oxygen content	0.31% (CV=43.9%, n=12)	0.25% (CV=62.69%, n=12)
Bacterial colony count (CFU/mL)	0 (pre-fill), 0 (post-lyophilisation)	0 (pre-fill), 0 (post-lyophilisation)
Mould/Yeast colony count (CFU/mL)	0 (pre-fill), 0 (post-lyophilisation)	0 (pre-fill), 0 (post-lyophilisation)

CV = coefficient of variation; n = number of samples tested; CFU = colony forming unit

Table 2. Accelerated degradation study for candidate standard 25/136

Time stored	Storage Temperature (°C)	Potency relative to -20°C	95% Confidence Limits	
2 weeks	+4	1.02	1.00	1.04
	+20	0.88	0.86	0.89
	+37	0.93	0.91	0.95
	+45	0.90	0.89	0.92
1 month	+4	1.13	1.09	1.16
	+20	1.22	1.18	1.25
	+37	1.20	1.17	1.24
	+45	1.08	1.05	1.11
3 months	+4	0.96	0.94	0.98
	+20	1.06	1.03	1.08
	+37	0.93	0.91	0.95
	+45	0.89	0.87	0.90
6 months	+4	0.97	0.95	1.00
	+20	0.90	0.88	0.92
	+37	0.87	0.85	0.89
	+45	0.79	0.77	0.80
12 months	+4	0.90	0.87	0.93
	+20	0.85	0.82	0.87
	+37	0.92	0.89	0.95
	+45	0.57	0.55	0.59

Table 3. Accelerated degradation study for candidate standard 25/168

Time stored	Storage Temperature (°C)	Potency relative to -20°C	95% Confidence Limits	
2 weeks	+4	0.88	0.85	0.90
	+20	0.96	0.93	0.98
	+37	1.00	0.97	1.02
	+45	1.08	1.06	1.11
1 month	+4	1.01	0.99	1.03
	+20	1.03	1.01	1.06
	+37	1.19	1.17	1.22
	+45	1.21	1.18	1.23
3 months	+4	1.00	0.98	1.03
	+20	1.05	1.03	1.08
	+37	1.12	1.09	1.15
	+45	1.16	1.14	1.19
6 months	+4	0.99	0.96	1.02
	+20	1.03	0.99	1.06
	+37	1.15	1.12	1.19
	+45	1.23	1.19	1.27
11 months	+4	0.98	0.93	1.02
	+20	1.04	0.99	1.08
	+37	1.19	1.14	1.25
	+45	1.28	1.22	1.33

Table 4: Homogeneity results for candidate preparations 25/136 and 25/168

Sample	Vial	Potency estimates as % of Vial 1	95% LCL	95% UCL
25/136	2	100.64	97.11	104.30
	3	102.11	99.47	104.82
	4	99.28	97.98	100.59
	5	102.44	96.13	109.18
	6	99.13	97.62	100.67
	7	100.64	96.54	104.92
	8	99.20	93.31	105.46
	9	96.92	93.70	100.25
	10	97.27	92.15	102.68
25/168	2	101.78	95.52	108.46
	3	100.89	93.87	108.42
	4	100.18	92.48	108.53
	5	99.68	95.41	104.13
	6	99.28	95.62	103.08
	7	99.88	93.78	106.38
	8	96.74	89.28	104.83
	9	95.47	78.05	116.78
	10	96.90	84.68	110.87

Table 5. Collaborative Study Sample Panel

Sample Study Code	Sample Type	Volume (mL)	Container
W1044	1st WHO IS (lyophilised)	0.5	DIN Ampoule
VZV-01	Immunoglobulin Candidate 2nd WHO IS (liquid frozen)	0.5	Eppendorf tubes
VZV-02	Immunoglobulin Candidate 2nd WHO IS (lyophilised)	0.5	DIN Ampoule
VZV-03	Human immunoglobulin (liquid frozen)	0.5	Eppendorf tubes
VZV-04			
VZV-05			
VZV-06	Plasma Candidate 2nd WHO IS (liquid frozen)	0.5	Eppendorf tubes
VZV-07	Plasma Candidate 2nd WHO IS (lyophilised)	0.5	DIN Ampoule
VZV-08	Plasma Candidate 2nd WHO IS (lyophilised)	0.5	DIN Ampoule
VZV-09	Human plasma (liquid frozen)	0.5	Eppendorf tubes
VZV-10			
VZV-11			
VZV-12			
VZV-13			
VZV-14			
VZV-15			
VZV-16			
VZV-17			
VZV-18			
VZV-19			
VZV-20			
VZV-21			
VZV-22			
VZV-23	Human serum (liquid frozen)	0.5	Eppendorf tubes
VZV-24			
VZV-25			
VZV-26			
VZV-27			
VZV-28			
VZV-29			
VZV-30			
VZV-31			
VZV-32	Depleted IgG/A human serum	0.5	Eppendorf tubes

Table 6. Laboratory codes and assay methods used

Lab	Assay	Format	Antibody detected	Target Antigen	Readout	Reported Result
1	SERION ELISA classic Varicella-Zoster Virus IgG	Indirect ELISA	IgG	VZV glycoprotein	OD	IU/mL
2a	Liaison VZV-IgG	CLIA	IgG	VZV glycoprotein	RLU	IU/mL
2b	SERION ELISA classic Varicella-Zoster Virus IgG	Indirect ELISA	IgG	VZV glycoprotein	OD	IU/mL
3	SERION ELISA classic Varicella Zoster Virus IgG	Indirect ELISA	IgG	VZV glycoprotein	OD	IU/mL
4	EUROIMMUN Anti-VZV ELISA 2.0 (IgG)	Indirect ELISA	IgG	Highly purified VZV proteins (strain “Ellen”) from infected, human fibroblasts	OD	IU/mL
5a	TESTLINE CLINICAL DIAGNOSTICS S.R.O. EIA VZV IgG	Indirect ELISA	IgG	Inactivated VZV antigen	OD	IU/mL
5b	TESTLINE CLINICAL DIAGNOSTICS S.R.O. CLIA VZV IgG	CLIA	IgG	Highly purified native antigen and VZV glycoprotein	RLU	IU/mL
6	SERION ELISA classic Varicella-Zoster Virus IgG	Indirect ELISA	IgG	VZV glycoprotein	OD	IU/mL
8	LIAISON VZV IgG HT	CLIA	IgG	VZV glycoprotein antigen (partially purified extract of infected cell cultures, Ellen strain)	RLU	IU/mL
10	Alegria® Anti-VZV IgG (ORG 914G)	Indirect ELISA	IgG	Purified VZV glycoprotein from virus cell culture	OD	mIU/mL
12a	In-house immunofluorescence assay	IFA	IgG	Whole virus antigen	Endpoint	+ / -
12b	SEIKEN anti-VZV ELISA antibody	Indirect ELISA	IgG	Whole virus antigen	OD	ELISA unit
13	LIAISON VZV IgG HT	CLIA	IgG	VZV glycoprotein antigen (partially purified extract of infected cell cultures, Ellen strain)	RLU	IU/mL
14	LIAISON VZV IgG HT	CLIA	IgG	VZV glycoprotein antigen (partially purified extract of infected cell cultures, Ellen strain)	RLU	IU/mL
15	SERION ELISA classic Varicella-Zoster Virus IgG	Indirect ELISA	IgG	VZV glycoprotein	OD	IU/mL
16	VIDAS Varicella-Zoster IgG	Direct ELISA	IgG	Varicella antigen	RFU	+ / -
17	In-house ELISA	Direct ELISA	IgG	gE from VZV	OD	mIU/mL
19	PLATELIA ELISA Kit	Indirect ELISA	IgG	Partially purified inactivated VZV	OD	+/-

OD = optical density; IU = International Unit; CLIA - chemiluminescent immunoassay; RLU = relative light units; RFU = relative fluorescence value
FAMA=Fluorescence-Antibody-to-Membrane-Antigen; IFA= Immunofluorescence assay

Table 7a: Potency estimates for samples VZV-01, VZV-02, VZV-06, VZV-07, and VZV-08, calculated relative to the current IS, W1044, in mIU/mL. GM= Geometric mean, GCV= Geometric coefficient of variation (%), UQ/LQ=interquartile range, N= number of valid estimates used in calculations, NP= test sample non-parallel to reference sample, NL= test sample non-linear, n/t=sample not tested by laboratory, n/a= not calculated as N < 3

Lab	VZV-01			VZV-02			VZV-06			VZV-07			VZV-08			VZV-07/VZV-08		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	33495	n/a	2	32199	n/a	2	4887	7.05	3	4604	7.46	3	4766	7.77	3	4684	7.07	6
02a	31495	6.24	3	30341	3.44	3	3619	n/a	2	4105	n/a	1	3787	1.73	3	3864	4.36	4
02b	35722	20.05	3	29115	15.55	3	3904	n/a	2	3031	n/a	2	3933	n/a	1	3306	33.13	3
03	31899	32.45	3	31758	29.62	3	3314	22.37	3	3115	36.12	3	3014	28.30	3	3064	28.58	6
04	18684	18.93	3	20213	2.11	3	1531	n/a	1		NL		1848	n/a	1	1848	n/a	1
05a	31800	n/a	2	32324	n/a	2		NP			NP			NL/NP			NL/NP	
05b	34156	n/a	2	33659	20.47	3	5717	n/a	2	6746	n/a	2	6640	n/a	2	6692	4.27	4
06		n/t		32201	14.06	11		n/t		4138	12.42	10		n/t		4138	12.42	10
08	35214	0.91	3	32307	1.57	3	4807	3.55	3	4587	3.36	3	4434	5.64	3	4510	4.56	6
10	44791	3.04	3	42938	10.73	3	3958	6.62	3	3844	5.43	3	3963	4.55	3	3903	4.79	6
13	38000	16.22	3	34637	5.99	3	5108	n/a	2	5693	n/a	2	5283	n/a	2	5484	4.64	4
15	30145	5.04	3	29137	7.99	6	2884	13.55	3	2877	11.86	6	2775	18.04	5	2830	14.21	11
16	44253	n/a	2	42079	n/a	2	6166	16.36	3		NL		5263	n/a	2	5263	n/a	2
17	21600	24.83	3	19715	25.67	3	6415	21.51	3	6143	21.50	3	6244	16.89	3	6193	17.13	6
19	48468	n/a	2	36764	8.34	6	4376	n/a	2	4297	18.26	4	3799	17.38	5	4013	18.07	9
GM	33277			31315			4115			4282			4071			4064		
Robust GM	34131			32150			4344			4257			4164			4163		
Median	33824			32201			4376			4216			3963			4075		
GCV	29.60			24.02			46.42			31.43			41.76			40.34		
UQ/LQ	1.19			1.15			1.41			1.33			1.39			1.49		
N	14			15			13			12			13			14		

Table 7b: Potency estimates for immunoglobulin samples VZV-03 to VZV-05, calculated relative to the current IS, W1044, in mIU/mL. GM= Geometric mean, GCV= Geometric coefficient of variation (%), UQ/LQ=interquartile range, N= number of valid estimates used in calculations, NP= test sample non-parallel to reference sample, NL= test sample non-linear, n/t=sample not tested by laboratory, n/a= not calculated as N < 3

Lab	VZV-03			VZV-04			VZV-05		
	GM	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**
01	15620	n/a	2	16751	n/a	2	19005	n/a	2
02a	13264	n/a	2	16678	n/a	2	19476	n/a	2
02b	15988	30.00	3	23484	21.46	3	26942	35.83	3
03	4124	42.65	3	16268	26.37	3	17721	25.05	3
04	7208	6.74	3	8685	11.15	3	9057	n/a	1
05a	NP			NL/NP			35200	n/a	1
05b	34363	n/a	2	35829	n/a	2	42517	29.12	2
06	14078	11.38	11	22271	5.00	5	20306	8.42	4
08	19271	6.45	3	28205	7.94	3	30940	2.15	3
10	14964	7.57	3	20938	2.34	3	25189	7.53	3
13	24796	24.14	3	31079	n/a	2	35131	n/a	2
15	12014	4.62	3	15963	3.76	3	19028	5.61	3
16	NL/NP			NL			43718	n/a	1
17	22584	28.55	3	31901	28.44	3	39727	20.86	3
19	16883	n/a	2	24946	n/a	2	30157	n/a	1
GM	14747			21179			25597		
Robust GM	15807			21792			26383		
Median	15620			22271			26942		
GCV	71.06			46.82			52.90		
UQ/LQ	1.45			1.69			1.83		
N	13			13			15		

Figure 1: Geometric mean potency estimates for samples VZV-01 to VZV-08 relative to the current IS, W1044, in mIU/mL, with 95% confidence intervals

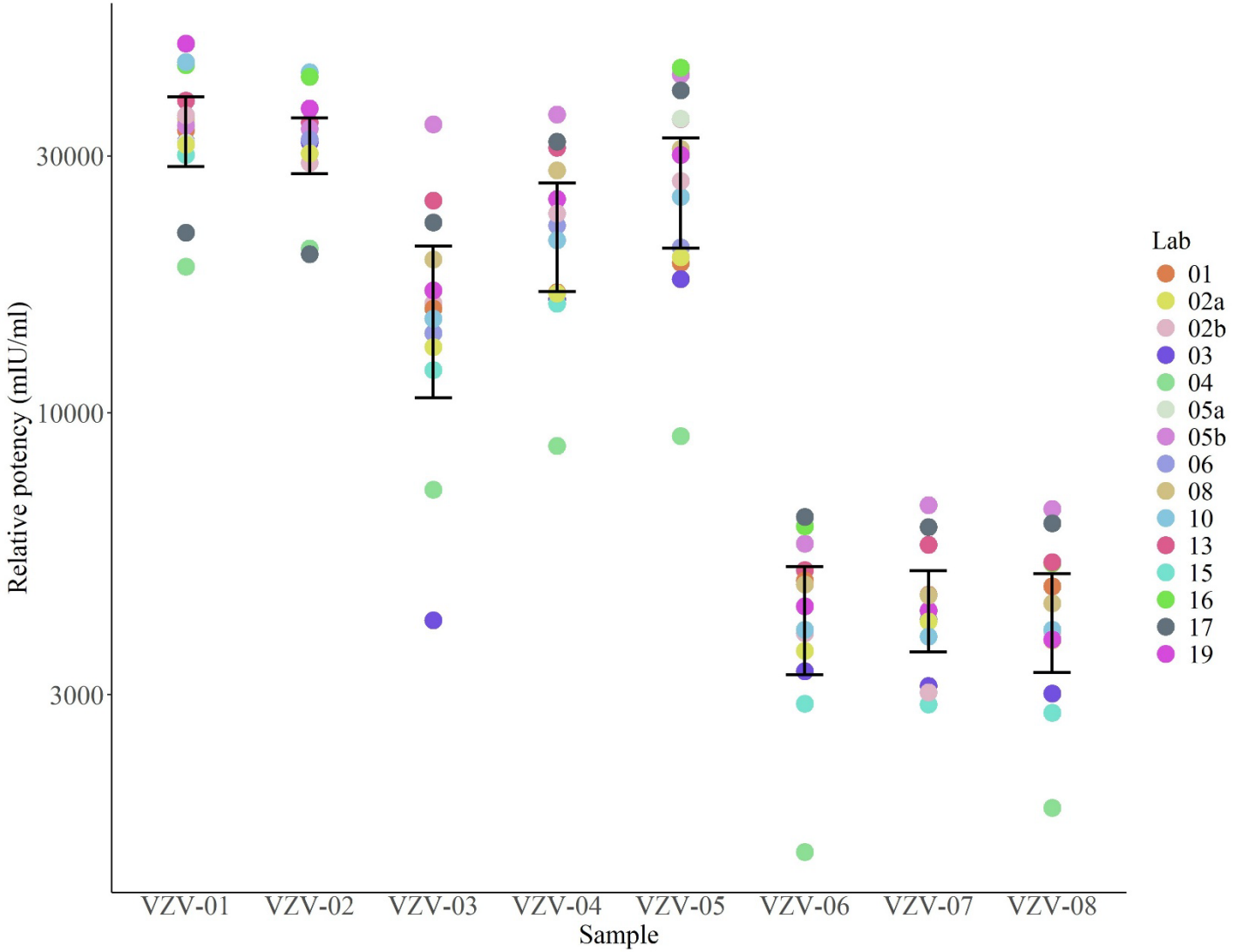


Table 8: Laboratory reported geometric mean estimates of study samples in mIU/mL. n/r = not reported by laboratory, n/t = not tested by laboratory, GM=geometric mean, GCV= Geometric coefficient of variation (%), UQ/LQ=interquartile range, N= number of valid estimates used in calculations

Lab	W1044	VZV-01	VZV-02	VZV-03	VZV-04	VZV-05	VZV-06	VZV-07	VZV-08
01	n/r	32710	30883	14772	18075	20963	4794	4513	4673
02a	61709	60152	41927	17017	21922	27554	4783	5331	5231
02b	57638	43211	34577	17577	26879	32751	4047	3483	3498
03	87106	57211	58134	7281	29244	31628	5997	5868	5792
04	103587	38641	44510	14617	19544	22886	3247	3612	3542
05b	4258	4363	4418	n/r	n/r	n/r	2340	2145	2201
06	41635	n/t	27602	11448	15676	17401	n/t	3224	n/t
08	48321	34278	31213	18162	26977	29048	4635	4411	4252
10	72413	64102	59626	20623	28896	32439	5555	5377	5696
13	52196	39766	36130	24517	38596	43537	4792	5281	5028
15	104158	64219	62126	25359	33269	40748	6161	6894	6106
17	41789	25647	24716	27971	37065	45559	7700	7721	7890
GM	49654	35644	32633	17035	25915	30066	4700	4565	4663
Robust GM	59720	41043	37315	17913	26188	30265	4851	4671	4781
Median	57638	39766	35345	17577	26977	31628	4792	4882	5028
GCV	140.68	113.79	101.41	47.35	34.28	35.66	38.29	42.90	41.11
UQ/LQ	1.77	1.75	1.58	1.53	1.51	1.45	1.33	1.54	1.48
N	11	11	12	11	11	11	11	12	11

Table 9: Potency estimates for immunoglobulin samples VZV-03 to VZV-05, expressed relative to the candidate IS, 25/168 (VZV-02), in mIU/mL. GM= Geometric mean, GCV= Geometric coefficient of variation (%), UQ/LQ=interquartile range, N= number of valid estimates used in calculations, NP= test sample non-parallel to reference sample, NL= test sample non-linear

Lab	Potency vs 25/168			% of potency vs W1044
	VZV-03	VZV-04	VZV-05	
01	15524	16647	18888	99.4%
02a	13990	17591	20542	105.5%
02b	17573	25812	29612	109.9%
03	4155	16391	17856	100.8%
04	11411	13749	14338	158.3%
05a	NP	NL/NP	34848	99.0%
05b	32669	34063	40421	95.1%
06	13990	22132	20179	99.4%
08	19088	27937	30646	99.0%
10	11152	15604	18772	74.5%
13	22909	28714	32457	92.4%
15	13195	17531	20897	109.8%
16	NL/NP	NL	33246	76.0%
17	36656	51779	64480	162.3%
19	14696	21713	26250	87.0%
GM	15454	22193	26157	
Robust GM	15736	21568	25558	
Median	14696	21713	26250	
GCV	71.52	45.40	47.70	
UQ/LQ	1.45	1.68	1.68	
N	13	13	15	

Table 10: Potency estimates for immunoglobulin samples VZV-03 to VZV-05, expressed relative to the candidate IS, 25/136 (VZV-07/VZV-08), in mIU/mL. GM= Geometric mean, GCV= Geometric coefficient of variation (%), UQ/LQ=interquartile range, N= number of valid estimates used in calculations, NP= test sample non-parallel to reference sample, NL= test sample non-linear

Lab	Potency vs 25/136			% of potency vs W1044
	VZV-03	VZV-04	VZV-05	
01	13338	14304	16229	85.4%
02a	13731	17266	20162	103.5%
02b	19346	28416	32600	121.0%
03	5384	21238	23135	130.6%
04	15601	18797	19603	216.4%
05a	NL/NP	NL/NP	NL/NP	n/a
05b	20538	21415	25412	59.8%
06	13610	21530	19631	96.7%
08	17092	25016	27441	88.7%
10	15336	21459	25816	102.5%
13	18087	22670	25626	72.9%
15	16981	22563	26895	141.3%
16	NL/NP	NL	33228	76.0%
17	14586	20603	25658	64.6%
19	16830	24867	30063	99.7%
GM	14807	21264	24628	
Robust GM	15757	21629	24820	
Median	15601	21459	25642	
GCV	39.43	18.90	22.96	
UQ/LQ	1.24	1.10	1.31	
N	13	13	14	

Table 11: Laboratory reported geometric mean estimates of immunoglobulin samples, VZV-03 to VZV-05, in mIU/mL, compared with potency estimates relative to the candidate IS, 25/168, in mIU/mL. GM= Geometric mean, GCV= Geometric coefficient of variation (%), UQ/LQ=interquartile range, N= number of valid estimates used in calculations, n/r=not reported by lab, n/a=unable to calculate

Lab	Laboratory reported estimates			Potency vs 25/168			Potency as % of reported		
	VZV-03	VZV-04	VZV-05	VZV-03	VZV-04	VZV-05	VZV-03	VZV-04	VZV-05
01	14772	18075	20963	15524	16647	18888	105.1%	92.1%	90.1%
02a	17017	21922	27554	13990	17591	20542	82.2%	80.2%	74.5%
02b	17577	26879	32751	17573	25812	29612	100.0%	96.0%	90.4%
03	7281	29244	31628	4155	16391	17856	57.1%	56.1%	56.5%
04	14617	19544	22886	11411	13749	14338	78.1%	70.3%	62.7%
05b	n/r	n/r	n/r	32669	34063	40421	n/a	n/a	n/a
06	11448	15676	17401	13990	22132	20179	122.2%	141.2%	116.0%
08	18162	26977	29048	19088	27937	30646	105.1%	103.6%	105.5%
10	20623	28896	32439	11152	15604	18772	54.1%	54.0%	57.9%
13	24517	38596	43537	22909	28714	32457	93.4%	74.4%	74.6%
15	25359	33269	40748	13195	17531	20897	52.0%	52.7%	51.3%
17	27971	37065	45559	36656	51779	64480	131.0%	139.7%	141.5%
GM	17035	25915	30066	15519	22233	25026			
Robust GM	17913	26188	30265	15870	21502	23968			
Median	17577	26977	31628	14737	19731	20719			
GCV	47.35	34.28	35.66	75.65	47.83	52.62			
UQ/LQ	1.53	1.51	1.45	1.57	1.70	1.65			
N	11	11	11	12	12	12			

Table 12: Laboratory reported geometric mean estimates of immunoglobulin samples, VZV-03 to VZV-05, in mIU/mL, compared with potency estimates relative to the candidate IS, 25/136, in mIU/mL. GM= Geometric mean, GCV= Geometric coefficient of variation (%), UQ/LQ=interquartile range, N= number of valid estimates used in calculations, n/r=not reported by lab, n/a=unable to calculate

Lab	Laboratory reported estimates			Potency vs 25/136			Potency as % of reported		
	VZV-03	VZV-04	VZV-05	VZV-03	VZV-04	VZV-05	VZV-03	VZV-04	VZV-05
01	14772	18075	20963	13338	14304	16229	90.3%	79.1%	77.4%
02a	17017	21922	27554	13731	17266	20162	80.7%	78.8%	73.2%
02b	17577	26879	32751	19346	28416	32600	110.1%	105.7%	99.5%
03	7281	29244	31628	5384	21238	23135	74.0%	72.6%	73.1%
04	14617	19544	22886	15601	18797	19603	106.7%	96.2%	85.7%
05b	n/r	n/r	n/r	20538	21415	25412	n/a	n/a	n/a
06	11448	15676	17401	13610	21530	19631	118.9%	137.3%	112.8%
08	18162	26977	29048	17092	25016	27441	94.1%	92.7%	94.5%
10	20623	28896	32439	15336	21459	25816	74.4%	74.3%	79.6%
13	24517	38596	43537	18087	22670	25626	73.8%	58.7%	58.9%
15	25359	33269	40748	16981	22563	26895	67.0%	67.8%	66.0%
17	27971	37065	45559	14586	20603	25658	52.1%	55.6%	56.3%
GM	17035	25915	30066	14650	20988	23625			
Robust GM	17913	26188	30265	15654	21317	23958			
Median	17577	26977	31628	15468	21437	25519			
GCV	47.35	34.28	35.66	41.17	19.01	21.16			
UQ/LQ	1.53	1.51	1.45	1.27	1.12	1.30			
N	11	11	11	12	12	12			

Table 13: Geometric mean estimates (mIU/mL) for immunoglobulin (top section), plasma (middle section) and serum (bottom section) samples calculated relative to the current IS, W1044. GM=geometric mean, GCV=geometric coefficient of variation, IQR=Inter-Quartile Range. Samples are listed by increasing median VZV concentration within each sample type (section).

Sample	Lab										GM	Median	GCV	IQR
	02a	02b	03	04	05b	08	10	13	15	17				
VZV-03	13264	15988	4124	7208	34363	19271	14964	24796	12014	22584	14533	15468	85.45	1.76
VZV-04	16678	23484	16268	8685	35829	28205	20938	31079	15963	31901	21222	22174	54.15	1.85
VZV-05	19476	26942	17721	9057	42517	30940	25189	35131	19028	39727	24377	26051	59.15	1.78
VZV-20	76	91	61	52	224	122	76	104	49	334	98	84	86.14	1.82
VZV-13	80	90	68	62	299	134	80	119	50	307	106	85	86.85	1.84
VZV-21	100	89	138	129	213	102	184	91	106	531	142	117	73.15	1.71
VZV-17	177	128	158	113	158	155	297	136	125	193	158	156	31.83	1.32
VZV-14	171	352	148	144	570	448	182	374	120	1734	293	253	128.70	2.78
VZV-22	192	381	167	168	539	477	186	382	134	642	282	270	77.69	2.62
VZV-19	289	256	262	197	309	291	399	254	203	357	276	276	24.83	1.20
VZV-10	305	241	276	195	491	363	3244	308	225	902	413	306	133.45	1.83
VZV-15	286	666	593	446	448	676	704	608	427	469	515	527	32.58	1.46
VZV-16	439	741	510	405	680	849	858	704	360	913	615	692	40.58	1.80
VZV-18	548	1182	591	564	808	1134	887	979	482	1100	787	847	40.39	1.87
VZV-12	943	2473	909	459	1092	2649	1046	2102	660	3595	1308	1069	95.40	2.59
VZV-09	2736	1674	1954	1663	957	1565	3153	1349	1622	843	1626	1642	50.30	1.34
VZV-11	2354	2733	1779	1241	1161	2577	2361	2226	1385	2271	1926	2248	37.23	1.60
VZV-31	157	111	147	103	155	160	139	104	116	260	140	143	32.18	1.40
VZV-23	251	308	243	174	469	436	285	334	178	641	306	297	51.81	1.67
VZV-25	258	376	237	119	568	482	338	300	191	914	325	319	78.40	1.87
VZV-26	402	491	456	274	575	644	529	473	348	626	467	482	30.72	1.36
VZV-29	403	1769	380	219	1223	1814	566	1291	323	2046	759	832	129.51	4.24
VZV-24	788	1884	970	720	935	1407	967	973	579	1949	1038	969	48.78	1.56
VZV-30	999	1318	1031	685	744	1476	1127	967	867	1218	1016	1015	27.51	1.34
VZV-27	838	3087	919	780	1567	4874	837	4868	750	11978	1868	1200	171.21	5.19
VZV-28	1686	1975	1687	1005	996	2100	2201	1717	1363	1710	1591	1699	32.20	1.33

Table 13a: Geometric mean estimates for immunoglobulin (top section), plasma (middle section) and serum (bottom section) samples calculated relative to the current IS, W1044, expressed as a % of the study median estimate; Shaded cells are outside the range of 55.3-180.9%. Samples are listed by increasing median VZV concentration within each sample type (section).

Sample	Lab									
	02a	**02b**	**03**	**04**	**05b**	**08**	**10**	**13**	**15**	**17**
VZV-03	85.8	103.4	26.7	46.6	222.2	124.6	96.7	160.3	77.7	146.0
VZV-04	75.2	105.9	73.4	39.2	161.6	127.2	94.4	140.2	72.0	143.9
VZV-05	74.8	103.4	68.0	34.8	163.2	118.8	96.7	134.9	73.0	152.5
VZV-20	91.5	109.3	72.8	62.6	268.3	145.4	90.7	124.6	58.5	399.2
VZV-13	93.7	105.6	79.9	72.6	351.5	157.2	94.7	140.6	58.7	361.4
VZV-21	85.1	75.5	118.0	110.2	181.2	87.0	157.1	78.0	90.7	452.6
VZV-17	113.0	82.0	100.8	72.0	101.0	99.2	190.1	86.9	79.9	123.1
VZV-14	67.6	138.9	58.7	56.8	225.3	176.9	72.0	147.9	47.4	685.0
VZV-22	70.9	141.0	61.7	62.0	199.5	176.6	68.8	141.4	49.8	237.7
VZV-19	105.0	92.9	95.2	71.4	112.3	105.5	144.7	92.3	73.8	129.6
VZV-10	99.6	78.7	90.1	63.7	160.3	118.6	1058.6	100.4	73.3	294.5
VZV-15	54.2	126.2	112.5	84.5	84.8	128.1	133.4	115.2	80.9	88.9
VZV-16	63.4	107.1	73.8	58.5	98.2	122.7	124.1	101.8	52.1	132.0
VZV-18	64.8	139.6	69.9	66.6	95.5	134.0	104.8	115.6	57.0	129.9
VZV-12	88.2	231.3	85.0	42.9	102.2	247.8	97.9	196.6	61.7	336.3
VZV-09	166.6	101.9	119.0	101.3	58.3	95.3	192.0	82.1	98.7	51.3
VZV-11	104.7	121.6	79.1	55.2	51.6	114.6	105.0	99.0	61.6	101.0
VZV-31	110.0	77.8	103.0	71.9	108.6	111.7	97.1	72.7	80.8	181.8
VZV-23	84.6	103.9	81.8	58.7	158.0	146.8	96.3	112.6	60.2	216.2
VZV-25	80.8	118.0	74.5	37.3	178.3	151.1	106.1	94.2	59.8	286.8
VZV-26	83.4	101.9	94.6	56.8	119.4	133.7	109.9	98.1	72.2	129.9
VZV-29	48.4	212.6	45.6	26.3	147.0	218.0	68.0	155.2	38.8	245.9
VZV-24	81.3	194.5	100.2	74.3	96.5	145.3	99.8	100.5	59.7	201.2
VZV-30	98.5	129.9	101.5	67.5	73.3	145.4	111.1	95.3	85.4	120.0
VZV-27	69.8	257.2	76.6	65.0	130.6	406.1	69.7	405.7	62.5	998.1
VZV-28	99.3	116.3	99.3	59.1	58.7	123.6	129.6	101.0	80.3	100.7

Table 13b: Summary estimates for immunoglobulin, plasma and serum samples calculated relative to the current IS, W1044, expressed as a % of the study median estimate

	Lab									
	02a	**02b**	**03**	**04**	**05b**	**08**	**10**	**13**	**15**	**17**
	Immunoglobulin									
GM	78.4	104.2	51.1	39.9	180.3	123.5	95.9	144.7	74.2	147.4
95% LCL	64.7	100.7	12.6	27.7	115.0	113.1	92.7	115.5	67.1	136.7
95% UCL	95.1	107.9	207.2	57.5	282.6	134.7	99.3	181.3	82.0	158.9
	Plasma									
GM	87.0	113.0	84.8	68.0	128.7	131.0	135.0	112.2	65.8	197.0
95% LCL	73.5	95.4	74.4	59.1	92.9	111.1	91.5	96.5	57.7	128.1
95% UCL	102.9	133.9	96.8	78.4	178.3	154.5	199.0	130.3	75.0	302.9
	Serum									
GM	82.1	135.7	84.1	54.9	112.6	161.9	96.7	118.5	65.0	212.2
95% LCL	68.2	100.2	68.7	42.2	85.1	120.0	82.2	80.6	54.0	126.2
95% UCL	98.7	183.9	102.8	71.4	148.9	218.6	113.9	174.3	78.3	356.9

Figure 2: Geometric mean estimates vs. current IS, W1044, expressed as a % of the study median estimate for (a, top) immunoglobulin samples, (b, middle) plasma samples, (c, bottom) serum samples.

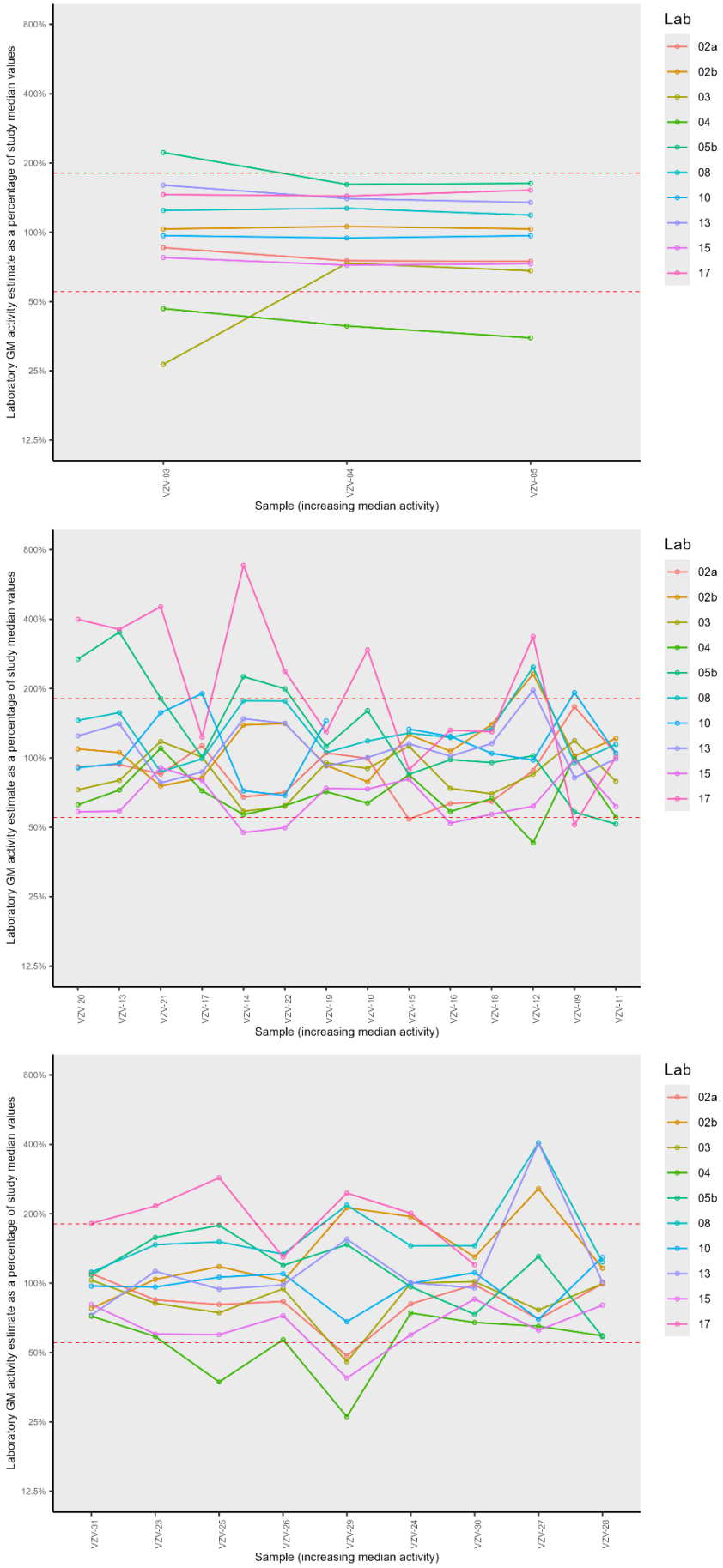


Table 14: Geometric mean estimates (mIU/mL) for immunoglobulin (top section), plasma (middle section) and serum (bottom section) samples calculated relative to the candidate IS, 25/168. GM=geometric mean, GCV=geometric coefficient of variation, IQR=Inter-Quartile Range. Samples are listed by increasing median VZV concentration within each sample type.

Sample	Lab										GM	Median	GCV	IQR
	02a	02b	03	04	05b	08	10	13	15	17				
VZV-03	13990	17573	4155	11411	32669	19088	11152	22909	13195	36656	15680	15679	86.21	1.85
VZV-04	17591	25812	16391	13749	34063	27937	15604	28714	17531	51779	22897	21308	52.21	1.71
VZV-05	20542	29612	17856	14338	40421	30646	18772	32457	20897	64480	26301	24876	56.71	1.67
VZV-20	81	100	61	83	213	120	56	96	54	541	105	89	102.37	1.75
VZV-13	84	99	68	98	284	132	60	110	55	498	114	98	100.91	1.76
VZV-21	105	97	139	205	202	101	137	84	117	862	153	127	96.58	1.80
VZV-17	186	141	159	178	150	154	222	126	137	313	170	156	30.95	1.29
VZV-14	181	386	150	228	542	444	136	346	132	2815	316	281	152.10	2.73
VZV-22	202	418	168	265	512	472	138	353	148	1042	304	306	91.29	2.61
VZV-19	305	281	264	311	294	288	297	235	223	580	297	291	29.57	1.13
VZV-10	322	265	278	309	467	360	2417	284	247	1465	445	315	120.20	1.56
VZV-15	301	732	598	706	426	670	524	562	469	761	555	579	33.45	1.44
VZV-16	463	814	514	641	646	841	640	651	396	1482	663	643	44.28	1.42
VZV-18	578	1299	596	893	768	1123	661	904	530	1785	849	828	48.04	1.74
VZV-12	995	2718	916	726	1039	2624	780	1942	725	5836	1412	1016	103.95	3.00
VZV-09	2885	1840	1969	2633	910	1550	2350	1246	1781	1367	1755	1810	42.73	1.59
VZV-11	2483	3004	1792	1965	1104	2552	1760	2057	1521	3686	2078	2010	41.60	1.43
VZV-31	166	122	148	163	148	158	103	96	127	422	151	148	50.33	1.31
VZV-23	265	339	245	276	445	431	213	309	196	1041	330	292	62.51	1.63
VZV-25	272	414	239	188	540	477	252	277	209	1484	351	275	85.21	1.90
VZV-26	424	540	459	433	547	638	394	437	382	1016	504	448	34.15	1.28
VZV-29	425	1944	382	347	1163	1797	422	1193	354	3321	819	703	133.62	4.14
VZV-24	831	2071	978	1139	888	1394	721	899	636	3163	1120	938	64.47	1.57
VZV-30	1054	1449	1038	1085	707	1462	840	893	952	1977	1096	1046	35.68	1.48
VZV-27	884	3393	926	1236	1490	4827	623	4498	823	19441	2016	1357	196.15	4.69
VZV-28	1779	2171	1700	1591	947	2080	1640	1586	1497	2776	1717	1670	32.45	1.26

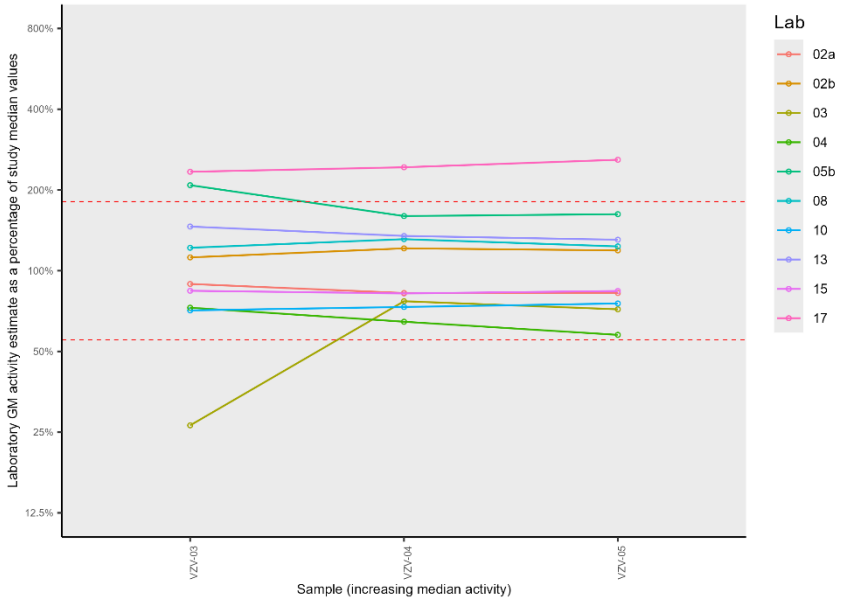
Table 14a: Geometric mean estimates for immunoglobulin (top section), plasma (middle section) and serum (bottom section) samples calculated relative to the candidate IS, 25/168, expressed as a % of the study median estimate. Shaded cells are outside the range of 55.3-180.9%. Samples are listed by increasing median VZV concentration within each sample type.

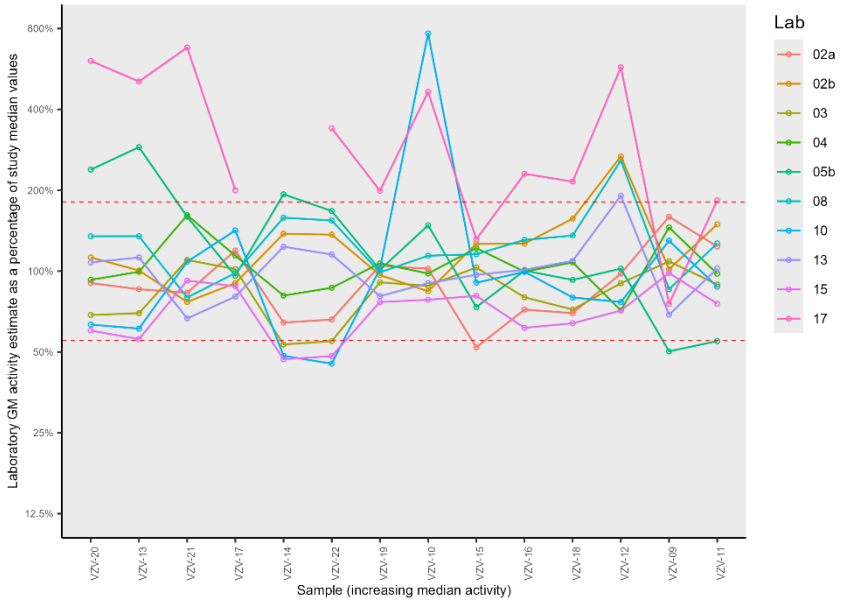
Sample	Lab									
	02a	**02b**	**03**	**04**	**05b**	**08**	**10**	**13**	**15**	**17**
VZV-03	89.2	112.1	26.5	72.8	208.4	121.7	71.1	146.1	84.2	233.8
VZV-04	82.6	121.1	76.9	64.5	159.9	131.1	73.2	134.8	82.3	243.0
VZV-05	82.6	119.0	71.8	57.6	162.5	123.2	75.5	130.5	84.0	259.2
VZV-20	90.3	112.5	68.6	92.8	238.7	134.8	63.2	107.8	60.1	606.5
VZV-13	85.6	100.5	69.8	99.5	289.4	134.9	61.1	112.5	55.9	508.0
VZV-21	83.1	76.8	110.1	161.6	159.5	79.8	108.4	66.7	92.2	680.1
VZV-17	119.3	90.2	101.7	114.1	96.1	98.3	141.8	80.4	87.8	200.1
VZV-14	64.4	137.8	53.3	81.1	193.3	158.1	48.4	123.3	47.0	1003.2
VZV-22	66.0	136.7	54.9	86.7	167.4	154.4	45.2	115.3	48.2	340.6
VZV-19	104.9	96.7	90.8	107.0	101.0	99.0	102.1	80.7	76.7	199.2
VZV-10	102.1	84.1	88.2	98.0	148.1	114.1	766.8	90.2	78.2	464.6
VZV-15	52.0	126.3	103.2	121.8	73.4	115.6	90.5	96.9	80.9	131.4
VZV-16	71.9	126.6	79.9	99.6	100.4	130.7	99.4	101.1	61.5	230.3
VZV-18	69.8	156.8	72.0	107.8	92.8	135.6	79.8	109.2	64.0	215.5
VZV-12	97.9	267.4	90.1	71.4	102.2	258.2	76.7	191.0	71.3	574.1
VZV-09	159.4	101.6	108.8	145.5	50.3	85.6	129.8	68.8	98.4	75.6
VZV-11	123.5	149.4	89.2	97.7	54.9	127.0	87.5	102.3	75.6	183.4
VZV-31	112.1	82.6	100.2	110.0	99.8	106.9	69.9	64.9	85.8	285.0
VZV-23	90.8	116.2	83.9	94.5	152.8	148.0	73.0	105.8	67.2	357.0
VZV-25	99.0	150.6	87.1	68.5	196.8	173.7	91.8	101.0	76.3	540.4
VZV-26	94.6	120.5	102.5	96.8	122.1	142.5	88.1	97.5	85.4	226.9
VZV-29	60.4	276.7	54.4	49.4	165.5	255.7	60.0	169.8	50.4	472.6
VZV-24	88.6	220.8	104.3	121.5	94.8	148.6	76.9	95.9	67.8	337.4
VZV-30	100.7	138.5	99.3	103.7	67.6	139.7	80.3	85.4	91.0	189.0
VZV-27	65.1	250.0	68.2	91.1	109.8	355.8	46.0	331.5	60.7	1432.9
VZV-28	106.5	130.0	101.8	95.2	56.7	124.6	98.2	95.0	89.7	166.2

Table 14b: Summary estimates for immunoglobulin, plasma and serum samples calculated relative to the candidate IS, 25/168, expressed as a % of the study median estimate.

	Lab									
	02a	**02b**	**03**	**04**	**05b**	**08**	**10**	**13**	**15**	**17**
	Immunoglobulin									
GM	84.7	117.4	52.7	64.7	175.6	125.3	73.3	137.0	83.5	245.1
95% LCL	75.8	106.0	12.0	48.4	121.4	113.5	68.1	118.5	80.9	215.3
95% UCL	94.7	129.9	231.8	86.4	253.9	138.3	78.8	158.3	86.1	279.0
	Plasma									
GM	88.3	119.6	82.3	103.7	117.8	124.9	96.8	99.8	69.6	307.9
95% LCL	74.2	99.5	71.8	91.6	87.2	105.5	65.2	85.6	60.9	202.4
95% UCL	105.1	143.7	94.2	117.4	159.1	148.0	143.8	116.3	79.5	468.3
	Serum									
GM	89.2	153.7	87.3	89.6	110.3	165.3	74.3	112.8	73.6	355.0
95% LCL	75.7	113.1	73.5	72.6	80.6	123.9	62.1	78.3	63.0	214.6
95% UCL	105.1	208.9	103.7	110.5	150.9	220.5	89.0	162.6	86.0	587.2

Figure 3: Geometric mean estimates vs. candidate IS, 25/168, expressed as a % of the study median estimate for (a, top) immunoglobulin samples, (b, middle) plasma samples, (c, bottom) serum samples.





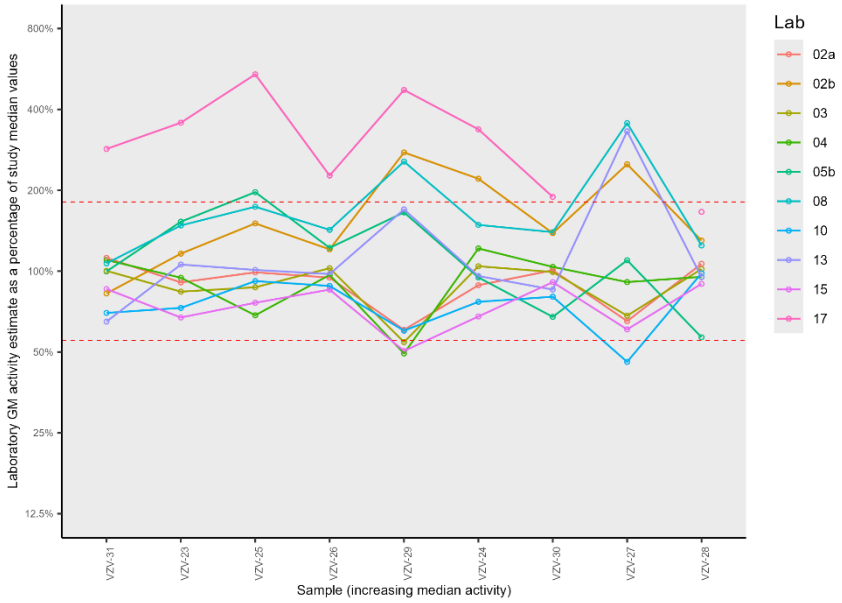


Table 15: Geometric mean estimates (mIU/mL) for immunoglobulin (top section), plasma (middle section) and serum (bottom section) samples calculated relative to the candidate IS, 25/136. GM=geometric mean, GCV=geometric coefficient of variation, IQR=Inter-Quartile Range. Samples are listed by increasing median VZV concentration within each sample type.

Sample	Lab										GM	Median	GCV	IQR
	02a	02b	03	04	05b	08	10	13	15	17				
VZV-03	13731	19346	5384	15601	20538	17092	15336	18087	16981	14586	14897	16277	46.04	1.21
VZV-04	17266	28416	21238	18797	21415	25016	21459	22670	22563	20603	21753	21437	14.85	1.09
VZV-05	20162	32600	23135	19603	25412	27441	25816	25626	26895	25658	24988	25642	16.01	1.12
VZV-20	79	111	79	113	134	108	78	76	69	215	100	93	41.65	1.44
VZV-13	82	109	89	133	179	118	82	87	71	198	108	98	41.57	1.55
VZV-17	183	155	206	244	94	138	305	99	177	124	162	166	45.74	1.57
VZV-21	103	107	181	280	127	91	189	67	150	343	145	138	66.54	1.79
VZV-19	300	310	342	426	185	258	409	185	287	231	283	293	33.71	1.41
VZV-14	177	425	194	311	341	397	187	273	170	1120	300	291	77.78	2.03
VZV-22	198	460	218	363	322	423	190	279	190	414	289	299	42.51	1.98
VZV-10	316	292	360	422	294	322	3324	224	317	583	423	320	115.31	1.36
VZV-15	296	805	775	965	268	600	721	443	604	303	528	602	59.40	2.28
VZV-16	454	896	666	876	406	753	880	514	509	590	630	627	33.98	1.65
VZV-18	567	1430	772	1221	483	1006	909	714	682	710	807	742	39.80	1.42
VZV-12	976	2993	1187	992	653	2350	1072	1533	933	2322	1341	1128	63.66	2.14
VZV-09	2832	2025	2551	3599	572	1388	3232	984	2292	544	1667	2155	100.28	2.57
VZV-11	2437	3307	2322	2686	694	2285	2420	1624	1957	1467	1974	2304	54.72	1.43
VZV-31	163	135	192	223	93	142	142	76	163	168	143	152	38.07	1.22
VZV-23	260	373	317	377	280	386	293	244	252	414	314	304	21.75	1.42
VZV-25	267	455	310	257	340	427	347	219	270	591	333	324	35.51	1.52
VZV-26	416	594	595	592	344	571	542	345	492	404	479	516	25.15	1.44
VZV-29	417	2140	495	474	731	1609	580	942	456	1321	778	651	79.54	2.53
VZV-30	1035	1595	1345	1483	444	1309	1155	705	1225	787	1042	1190	48.72	1.59
VZV-24	816	2280	1267	1557	559	1248	991	710	818	1259	1064	1112	51.10	1.55
VZV-27	868	3735	1200	1689	937	4323	857	3551	1060	7736	1915	1424	123.30	3.82
VZV-28	1746	2390	2203	2174	596	1863	2256	1252	1927	1105	1631	1895	54.66	1.61

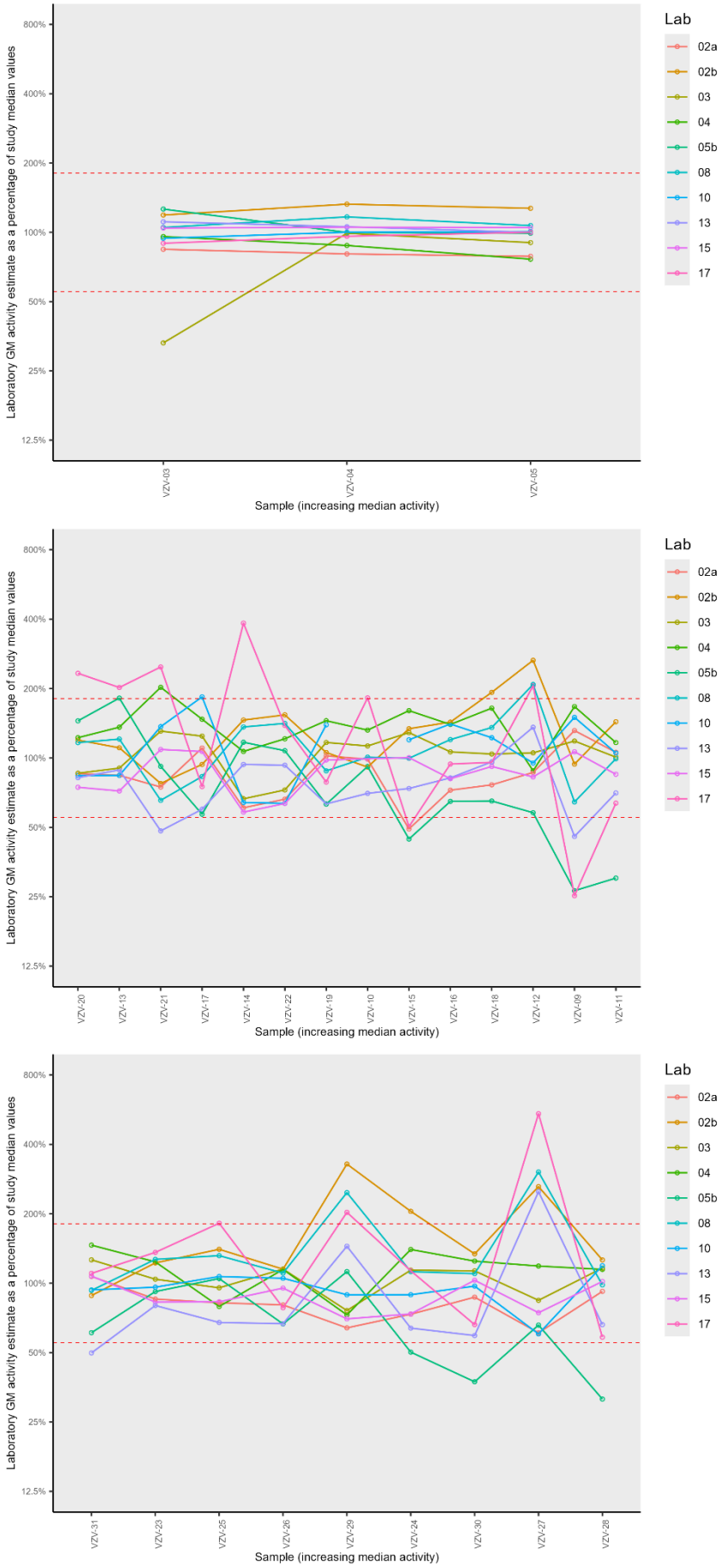
Table 15a: Geometric mean estimates for immunoglobulin (top section), plasma (middle section) and serum (bottom section) samples calculated relative to the candidate IS, 25/136, expressed as a % of the study median estimate. Shaded cells are outside the range of 55.3-180.9%. Samples are listed by increasing median VZV concentration within each sample type.

Sample	Lab									
	02a	**02b**	**03**	**04**	**05b**	**08**	**10**	**13**	**15**	**17**
VZV-03	81.6	114.9	32.0	92.7	122.0	101.6	91.1	107.5	100.9	86.7
VZV-04	80.5	132.4	99.0	87.6	99.8	116.6	100.0	105.6	105.1	96.0
VZV-05	78.6	127.1	90.2	76.4	99.0	107.0	100.6	99.9	104.8	100.0
VZV-20	85.5	119.5	85.8	122.4	144.8	116.5	83.9	82.1	74.6	232.9
VZV-13	84.0	110.7	90.4	136.0	181.9	120.7	84.0	88.8	71.9	202.1
VZV-21	74.8	77.5	130.7	202.5	91.9	65.5	136.7	48.3	108.8	248.1
VZV-17	110.5	93.7	124.4	147.2	57.0	83.1	184.0	59.9	106.7	75.1
VZV-14	60.8	146.0	66.5	106.7	117.0	136.3	64.1	93.7	58.2	384.2
VZV-22	66.2	153.8	72.6	121.1	107.5	141.2	63.5	93.0	63.4	138.4
VZV-19	102.1	105.6	116.7	145.0	63.0	87.9	139.2	63.2	98.0	78.6
VZV-10	98.8	91.3	112.7	132.0	91.8	100.8	1039.6	70.2	99.2	182.3
VZV-15	49.2	133.9	128.8	160.4	44.5	99.7	119.9	73.7	100.3	50.4
VZV-16	72.5	143.0	106.3	139.7	64.8	120.1	140.4	82.0	81.2	94.1
VZV-18	76.4	192.6	104.0	164.4	65.1	135.5	122.4	96.1	91.9	95.7
VZV-12	86.5	265.2	105.2	88.0	57.9	208.3	95.0	135.9	82.7	205.8
VZV-09	131.4	94.0	118.4	167.1	26.5	64.4	150.0	45.7	106.4	25.3
VZV-11	105.8	143.6	100.8	116.6	30.1	99.2	105.0	70.5	85.0	63.7
VZV-31	107.0	88.4	126.3	146.2	61.0	93.0	93.5	49.8	107.3	110.3
VZV-23	85.3	122.5	104.1	123.7	92.0	126.9	96.1	80.0	82.8	136.0
VZV-25	82.2	140.3	95.5	79.3	104.7	131.6	106.9	67.5	83.1	182.0
VZV-26	80.5	115.0	115.2	114.7	66.5	110.6	105.0	66.7	95.2	78.2
VZV-29	64.0	328.6	76.1	72.9	112.3	247.1	89.1	144.6	70.0	202.9
VZV-24	73.3	205.0	113.9	140.0	50.2	112.2	89.1	63.8	73.6	113.2
VZV-30	87.0	134.1	113.1	124.7	37.3	110.0	97.1	59.3	103.0	66.1
VZV-27	60.9	262.4	84.3	118.7	65.8	303.6	60.2	249.4	74.4	543.4
VZV-28	92.1	126.2	116.3	114.8	31.4	98.3	119.1	66.1	101.7	58.3

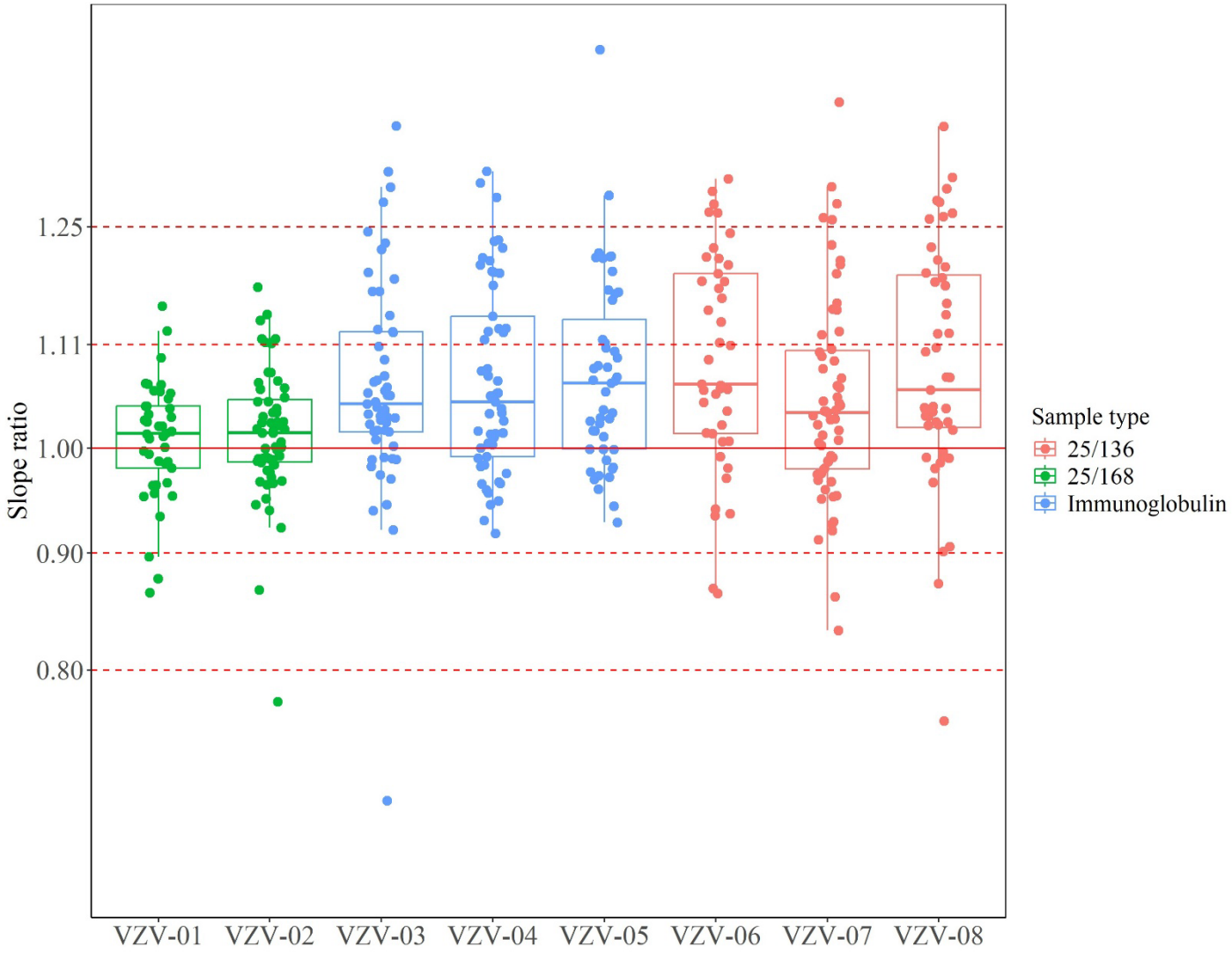
Table 15b: Summary estimates for immunoglobulin, plasma and serum samples calculated relative to the candidate IS, 25/136, expressed as a % of the study median estimate.

	Lab									
	02a	**02b**	**03**	**04**	**05b**	**08**	**10**	**13**	**15**	**17**
	Immunoglobulin									
GM	80.2	124.6	65.8	85.3	106.4	108.2	97.1	104.3	103.6	94.1
95% LCL	76.5	104.0	13.9	66.6	79.3	91.0	84.6	94.8	97.9	78.3
95% UCL	84.1	149.3	312.5	109.2	142.8	128.6	111.5	114.7	109.7	113.0
	Plasma									
GM	83.4	126.7	102.6	136.4	71.3	107.7	128.2	75.8	86.2	117.9
95% LCL	71.8	104.9	91.1	120.9	51.8	89.8	86.5	64.2	76.8	76.8
95% UCL	97.0	153.1	115.5	154.0	98.1	129.1	189.9	89.5	96.7	181.0
	Serum									
GM	80.3	155.2	103.7	112.3	63.6	135.7	93.7	81.7	86.9	129.5
95% LCL	70.1	111.8	91.2	93.6	45.3	98.6	81.0	55.1	76.7	76.5
95% UCL	91.9	215.3	118.0	134.8	89.2	186.8	108.5	121.2	98.4	219.5

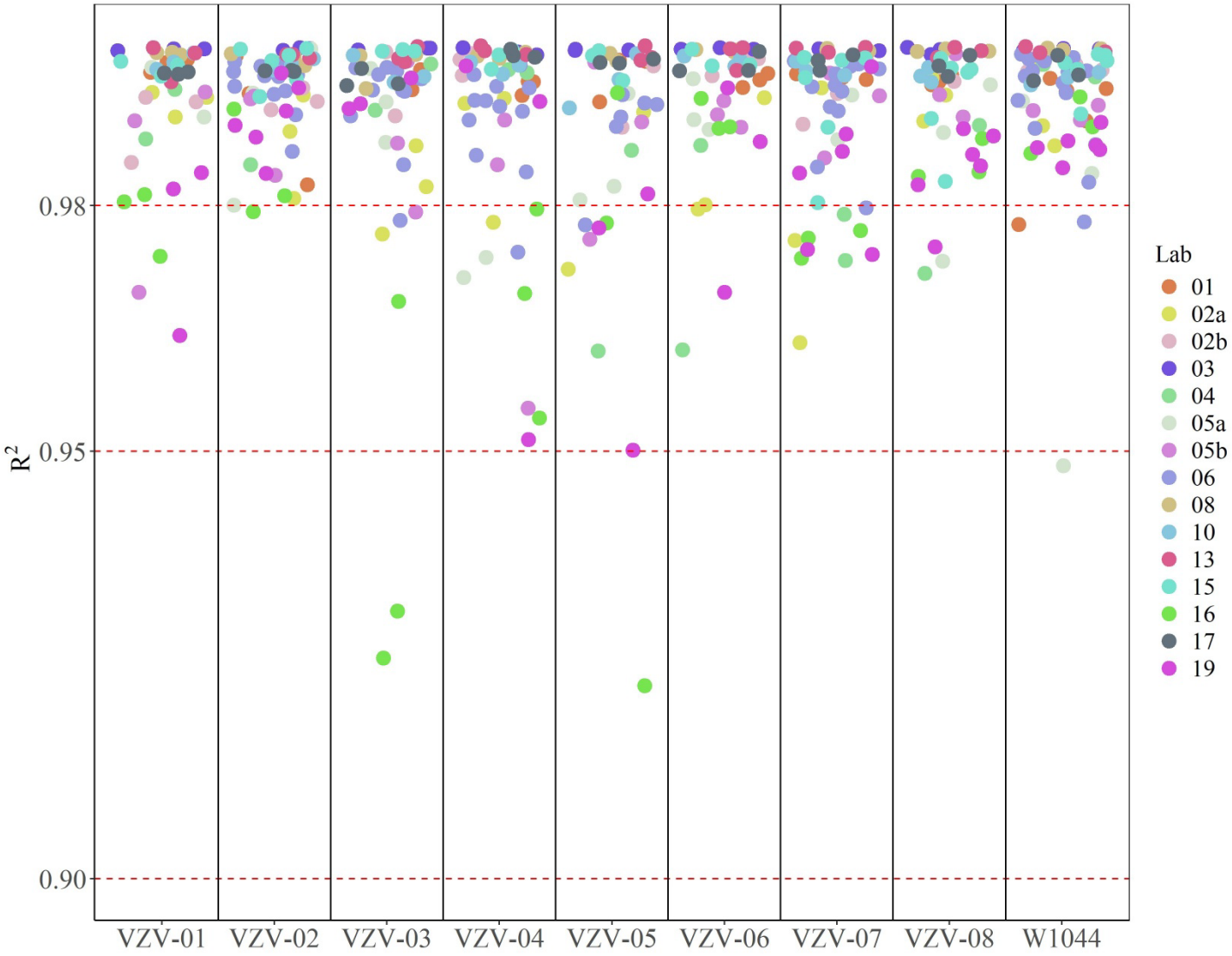
Figure 4: Geometric mean estimates vs. candidate IS, 25/136, expressed as a % of the study median estimate for (a, top) immunoglobulin samples, (b, middle) plasma samples, (c, bottom) serum samples.



Supplementary Figure 1: Slope ratios for samples VZV-01 to VZV-08 relative to the current standard, W1044



Supplementary Figure 2: R^2 values for all samples



Supplementary Table 1: Individual assay potency estimates relative to the current IS, W1044, in mIU/mL; NL=non-linear; NP=non-parallel; Std NL=reference standard non-linear

Lab	Assay	Plate	VZV-01	VZV-02	VZV-03	VZV-04	VZV-05	VZV-06	VZV-07	VZV-08
01	1	1	34852	32181	15994	22934	24475	-	-	-
01	2	1	32191	32217	15255	12235	14758	-	-	-
01	3	1	Std NL	Std NL	Std NL	Std NL	Std NL	-	-	-
01	5	1	-	-	-	-	-	5267	4952	5162
01	6	1	-	-	-	-	-	4807	4596	4712
01	7	1	-	-	-	-	-	4609	4289	4450
02a	1	1	29676	29236	NL	15899	NL	NL	NL	3713
02a	2	1	33491	31235	12358	NL	20936	3815	4105	3824
02a	3	1	31434	30585	14237	17496	18118	3434	NL	3825
02b	1	1	30406	24668	11822	20205	19445	NP	2376	NP
02b	2	1	34410	32162	18989	21916	28171	3885	3866	3933
02b	3	1	43569	31107	18207	29249	35700	3923	NP	NP
03	1	1	42994	41427	6033	21091	22448	4132	4275	3847
03	2	1	24608	24670	2986	13391	14393	2781	2309	2338
03	3	1	30680	31342	3894	15243	17224	3166	3062	3044
04	1	1	21122	19918	7548	9359	NL	1531	NL	1848
04	2	1	16528	20514	6883	8059	9057	NL	NL	NL
04	3	1	-	-	-	-	-	-	-	-
05a	1	1	31484	30310	NP	NL	35200	NP	NP	NP
05a	2	1	32119	34471	NP	NP	NP	NP	NP	NL/NP
05a	3	1	Std NL	Std NL	Std NL	Std NL	Std NL	Std NL	Std NL	Std NL
05b	1	1	31323	27306	19456	27139	35487	NP	NP	NP
05b	2	1	37245	39016	60693	47303	50939	6026	7026	6834
05b	3	1	NL	35794	NL	NL	NL	5424	6476	6451
06	1	1	-	-	16436	23974	-	-	3826	-
06	1	2	-	-	16155	20978	-	-	NL	-

06	1	3	-	31521	-	-	20827	-	3694	-
06	1	4	-	29468	-	-	19060	-	3413	-
06	1	5	-	28343	NL	2852	-	-	-	-
06	1	6	-	35292	14048	3446	-	-	-	-
06	2	1	-	-	15537	22559	-	-	4015	-
06	2	2	-	-	13871	21932	-	-	3794	-
06	2	3	-	Std NL	-	-	Std NL	-	Std NL	-
06	2	4	-	44568	-	-	22517	-	4873	-
06	2	5	-	27951	12334	3869	-	-	-	-
06	2	6	-	30844	12477	4118	-	-	-	-
06	3	1	-	-	13892	22017	-	-	4401	-
06	3	2	-	-	15521	NL	-	-	4695	-
06	3	3	-	33756	-	-	19021	-	4563	-
06	3	4	-	29314	-	-	NL	-	4356	-
06	3	5	-	32746	12507	3874	-	-	-	-
06	3	6	-	33365	12906	2917	-	-	-	-
08	1	1	34880	31745	17967	25888	30205	5001	4712	4275
08	2	1	35249	32708	20263	28911	31175	4681	4421	4318
08	3	1	35517	32477	19657	29981	31455	4745	4634	4722
10	1	1	46062	39192	14124	20995	23189	3796	3616	3906
10	2	1	44952	42142	16243	20433	26557	4261	3963	3825
10	3	1	43399	47932	14605	21396	25952	3832	3963	4166
13	1	1	31954	33952	31553	NP	NP	NP	NP	NP
13	2	1	41783	33095	23259	29058	36562	5736	5630	5350
13	3	1	41099	36981	20774	33241	33756	4548	5756	5216
15	1	1	31859	28595	12639	15356	18801	3298	3137	3486
15	1	2	-	31807	-	-	-	-	3145	3060
15	2	1	29658	31712	11837	16527	20195	2840	2732	2516
15	2	2	-	29553	-	-	-	-	2451	2285
15	3	1	28993	26662	11592	16027	18144	2561	2661	2682
15	3	2	-	26925	-	-	-	-	3224	NP

16	1	1	NL	NL	NL/NP	NL	43718	6721	NL	NP
16	2	1	41873	39581	NL	NL	NL	5176	NL	5272
16	3	1	46768	44735	NL	NL	NL	6738	NL	5253
17	1	1	20269	18012	19575	30784	38421	6191	5937	6545
17	2	1	17988	16642	19497	25331	33497	5387	5154	5246
17	3	1	27642	25566	30181	41632	48715	7915	7575	7091
19	1	1	50626	36166	17536	NL	NL	4104	NL	3179
19	1	2	-	37318	-	-	-	-	3451	3255
19	2	1	46403	41376	16254	27167	NL	4667	4903	4040
19	2	2	-	37834	-	-	-	-	NL	4108
19	3	1	NL	32334	NP	22905	30157	NL	4114	NL/NP
19	3	2	-	36142	-	-	-	-	4895	4607

Appendix 1. Collaborative Study Participants (in alphabetical order by country)

Participant	Organisation	Country
Nathalie Baudson, Valérie Berthold, Raphaël Serckx, Aksel Boudina	GlaxoSmithKline Biologicals SA - Belgium	Belgium
Lenka Hanáková, Markéta Burdová	TestLine Clinical Diagnostics s.r.o.	Czech Republic
Nathalie Auberger Ripoll	BioMerieux	France
Sabine Gardon, Inga Conrad-Kanofsky, Heike Bochtler	Biotest AG	Germany
Julia M. Klemens	Euroimmun Medizinische Labordiagnostika AG	Germany
Stefan Boeckler, Frank Krieger, Christina Loewel, Thomas Schumacher	Institut Virion\Serion GmbH	Germany
Anna Klemm, Cristian Gurgui, Karin Koralewski, Simon Alexander Borkowsky, Andrea Schnatz, Friede Nickelsen, Susanne Müller	Orgentec by Sebia	Germany
Lilija Miller	Paul-Ehrlich Institut	Germany
Daniela Huzly	University Medical Center Freiburg	Germany
Francesco Capuano, Ginevra Di Matteo	Diasorin Italia S.p.A.	Italy
Shuetsu Fukushi	National Institute of Infectious Diseases (NIID), Japan Institute for Health Security (JIHS)	Japan
Chitra Tejpal	Medicines and Healthcare products Regulatory Agency (MHRA)	UK
Hayley Bally, Jade Sharp	UKHSA Bristol	UK
Heather Lawson, Mihaela Cirdei	UKHSA Colindale	UK
James Barnes, Lewis Roden, Stephen Phoenix, Nathan Tims	UKHSA Manchester	UK

Appendix 2. Study protocol provided to study participants

Medicines & Healthcare products Regulatory Agency

STUDY PROTOCOL

Collaborative Study CS744
Development of the 2nd WHO International Standard for anti-Varicella Zoster virus Immunoglobulin

Study Background and Aims

This multi-center International collaborative study will evaluate a candidate preparation to serve as the 2nd WHO International Standard (IS) for anti-Varicella Zoster virus Immunoglobulin. This will replace the current 1st WHO International Standard (NIBSC product code: W1044) which was established by WHO in 1987 [1]. The study is organized by the MHRA on behalf of the World Health Organization (WHO).

International Standards are recognized as the highest order of reference material for biological substances and are assigned a potency in International Units (IU). They are intended to be used to calibrate assays to report the biological activity of samples or products in terms of IU. This allows for comparability between assays/laboratories and enables parameters, such as analytical sensitivity of tests and the measurement of antibody levels in immunoglobulin preparations or clinical measures of immunity, to be better defined. Replacement IS are intended to maintain continuity in the calibration and reporting of results in IU. There is also a requirement to ensure the candidate replacement remains fit-for-purpose, accounting for advancements in technology and the clinical landscape since the current WHO IS was established. This study will evaluate these factors following published WHO guidelines [2] and be submitted for formal establishment by WHO.

The aims of this study are to:

- Evaluate the candidate replacement's potency/readout, in parallel to the 1st WHO International Standard, in a range of typical assays performed in different laboratories.
- Characterise the candidate's reactivity/specificity in different assay systems.
- Assess commutability, i.e. establish the extent to which the candidate is suitable to serve as a standard for a variety of different samples.
- Propose a unitage for the candidate 2nd WHO International Standard that will ensure continuity in the use of the International Unit.

Study Samples

All study samples are provided coded and blinded to participants, along with the current WHO IS (W1044) (Table 1.). Each participant is provided with 4 study sample panels to allow for 3 independent tests per method, plus one spare which can be used for a preliminary assay. Laboratories with more than one method will receive additional sample sets.

The study samples (VZV-01 to VZV-32) comprise plasma, sera or purified immunoglobulin of human origin which have been collected from healthy donors. All donations have been screened and tested negative for blood borne pathogen markers HBsAg, anti-HIV Ab and HCV RNA. Plasma/sera samples have been clarified by centrifugation and filtration.

The current WHO IS (W1044) is a purified immunoglobulin of human origin. It has been tested and found negative for HBsAg, HCV RNA and HIV RNA. However, testing for anti-HIV Ab provides an indeterminate result and therefore the sample is provided as potentially infectious.

Table 1. CS744 Study Sample Panel.

All samples should be stored at -20°C or below.

*Further details of the testing requirements are provided in Section 2 of the Study Protocol

Sample Code	Formulation	Volume (mL)	Vial	Testing Protocol*
W1044	Freeze-dried	1.0	DIN Ampoule	Dilutions beyond endpoint
VZV-01	Liquid frozen	0.5	Screw Cap Vial	
VZV-02	Freeze-dried		DIN Ampoule	
VZV-03	Liquid frozen		Screw Cap Vial	
VZV-04				
VZV-05				
VZV-06				
VZV-07	Freeze-dried		DIN Ampoule	
VZV-08				
VZV-09	Liquid frozen	0.5	Screw Cap Vial	As per local, routine sample testing, SOP
VZV-10				
VZV-11				
VZV-12				
VZV-13				
VZV-14				
VZV-15				
VZV-16				
VZV-17				
VZV-18				
VZV-19				
VZV-20				
VZV-21				
VZV-22				
VZV-23				
VZV-24				
VZV-25				
VZV-26				
VZV-27				

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VZV-28				
VZV-29				
VZV-30				
VZV-31				
VZV-32				

CAUTION: As with all materials of biological origin, the material should be regarded as potentially hazardous to health.

Study Protocol

1. Sample Preparation

As per the Instructions for Use (IFU), prior to assay the freeze-dried samples W1044, VZV-02, VZV-07 and VZV-08 require reconstitution.

- W1044* should be reconstituted in 1.0mL of sterile distilled H_2O.
- VZV-02, VZV-07 and VZV-08 should be reconstituted in 0.5mL of sterile distilled H_2O.

Complete dissolution may take 10-20 minutes and require gentle mixing. Reconstitution should be performed at room temperature.

**To assist opening ampoules of W1044, score the stress point, where the narrow ampoule stem joins the wider ampoule body, using a blunt-ended blade.*

All other samples (VZV-01, VZV-03 to VZV-06 and VZV-09 to VZV-32) are provided liquid frozen and should be thawed at room temperature prior to assay.

2. Sample Testing

All participants are requested to:

- Perform 3 independent tests on different days or with different operators.
- Use a fresh set of samples for each independent test, ideally testing all samples within the same assay run/plate. If not possible, please prioritise samples W1044 and VZV-01 to VZV-08 for concurrent testing and include freeze-dried samples W1044, VZV-02 and VZV-07 and -08 within additional assay runs/plates. Please indicate in the results reporting sheet if samples have not been tested concurrently.
- Test samples in duplicate.
- Where required, prepare dilutions of the samples in the buffer or sample matrix stated in your local SOP.
- Include all routine assay controls/calibrants/references when performing testing and report any anomalies in test performance.

2.2 Quantitative Methods

For the purpose of this study, this includes all test methods which can provide quantitative or semi-quantitative results for analysis of sample potency (i.e. results reported in IU/mL).

Samples W1044 and VZV-01 to VZV-08:

- Prepare serial dilutions of samples to include at least 4 positive points, with at least one point beyond the endpoint dilution.
- It is recommended to set initial dilutions of samples VZV-01 to VZV-05 based on the potency of W1044 (50 IU/mL). It is recommended to use an initial dilution 10x less for samples VSV-06 to VZV-08.

- If needed, dilutions can be adjusted for subsequent assays and recorded clearly in the results reporting sheet.

Samples VZV-09 to VZV-32:

- Test samples according to routine sample testing procedure, to provide a quantitative sample result.
- Where samples record a result above the assay cut-off, if the testing procedure allows, please repeat the test after diluting the sample to report an absolute result. Record in the results reporting sheet where samples have been diluted.

2.3 Qualitative Methods

This includes test methods which cannot be used to quantify the potency of samples (i.e. results reported as pos/neg).

Samples W1044 and VZV-01 to VZV-08:

- Prepare serial dilutions of samples to include at least 2 positive points, with at least one point beyond the endpoint dilution.
- It is recommended to test samples using 2-fold dilutions.
- It is recommended to set initial dilutions of samples VZV-01 to VZV-05 based on the potency of W1044 (50 IU/mL). It is recommended to use an initial dilution 10x less for samples VSV-06 to VZV-08.
- If needed, dilutions can be adjusted for subsequent assays and recorded clearly in the results reporting sheet.

Samples VZV-09 to VZV-32:

- Test samples according to routine sample testing procedure, reporting results as positive/negative.

Results Reporting

An Excel reporting sheet is provided so that all essential information can be recorded, including details of test method and the raw data obtained from each assay. The use of the reporting sheet facilitates the consistent analysis and interpretation of results.

- Under the 'Result Summary' record the qualitative (+/-) and where applicable quantitative result for all samples tested, as per analysis in your laboratory. It is important to know whether the samples are considered positive and provide any additional comments on the sample result which will support data analysis.
- Under the 'Raw data' record the raw assay readout (e.g. absorbance OD, RLU, etc.) and dilutions tested for samples W1044, VZV-01 to VZV-08. For quantitative methods, record where samples VZV-09 to VZV-32 have been diluted for testing. Include the assay cut-off value indicating sero-reactivity for each assay.
- Where multiple methods have been used, complete one reporting sheet per method.
- Record in the Excel reporting sheet any deviations from the study protocol and complete all fields requesting additional method details.

Deadline for return of results is 6 weeks from date of receipt of samples

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All completed results spreadsheets should be returned electronically to:

Dr Daniella Di Mascio Daniella.diMascio@mhra.gov.uk
Viral Vaccine Standards Team virvac@mhra.gov.uk

Data Analysis

Analysis of the study data will assess the potencies of each of the study samples VZV-01 to VZV-08 relative to each other and the current WHO IS (W1044), and the interpretation of the other study samples VZV-09 to VZV-32 will be used to evaluate the performance of the samples within the different assay methods. The anonymity of each laboratory is maintained by reporting datasets with a blind code, details of the assay performed will be included.

A draft study report will be sent to participants for comment. The report will include data analysis, proposed conclusions and recommendations on the use and unitage of the candidate 2nd WHO International Standard for anti-Varicella Zoster virus Immunoglobulin. Participants' comments will be included in the report prior to submission to WHO. Study participants will be notified of the outcome of the study after the WHO ECBS meeting.

References

[1] WHO Expert Committee on Biological Standardization 38th Report, WHO Technical Report Series, No. 771. 1988. ISBN: 924120771X Available from: www.who.int/publications/i/item/924120771X

[2] WHO, Recommendations for the preparation, characterization and establishment of international and other biological reference standards. WHO Technical Report Series, No. 932., in Expert Committee on Biological Standardization. 2006.

As outlined in the study questionnaire, participation in the collaborative study is conducted under the following WHO Collaborative Study Terms and Conditions:

By joining the WHO International collaborative study to establish the 2nd WHO International Standard for anti-Varicella Zoster virus immunoglobulin

The participant agrees that:

- The study samples have been prepared from Materials provided by donors and therefore must be treated as proprietary;
- The Materials provided must not be shared with anyone outside of the study;
- The Materials must not be used for application in human subjects or animals in the human food chain in any manner or form;
- There must be no attempt to reverse engineer, ascertain the chemical structure of, modify, or make derivatives of, any of the Materials;
- Participants accept responsibility for safe handling and disposal of the materials provided in according to the local regulations in their organization/country.

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- Data obtained through testing of the Materials must not be published or cited before the formal establishment of the standard by World Health Organization, without the express permission of the MHRA study organiser.

MHRA, as the Collaborative Study coordinator, notes that:

- It is normal practice to acknowledge all participants as contributors of data rather than co-authors in publications;
- Data published from participating labs will be anonymised;
- Participation of this study is at your discretion and does not include remuneration costs;
- Prior to the establishment of the standard MHRA reserves the right to disclose specific information about the use of the Material(s), without acknowledgement of the study participants;
- Participants will receive a copy of the report of the study with proposed conclusions and recommendations for comment before it is further distributed.

Appendix 3. Draft Instructions for Use

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WHO International Standard
2nd WHO International Standard for Antibodies to Varicella Zoster Virus
NIBSC code: 25/168
Instructions for use
(Version0.1, Dated 04/09/2026)

1. INTENDED USE
The 2nd WHO International Standard for Varicella Zoster Immunoglobulin is intended for the calibration of immunoassays that measure anti-VZV antibody levels in human serum or plasma.

2. CAUTION
This preparation is not for administration to humans or animals in the human food chain.
The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE
16 IU/ampoule

4. CONTENTS
Country of origin of biological material: Germany
Each ampoule contains the freeze-dried reidue of a 0.5 mL fill of 5% human immunoglobulin preparation.

5. STORAGE
The recommended storage temperature is -20 °C.
Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

8. STABILITY
Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

9. REFERENCES
A comprehensive report of the production and characterisation of this standard has been published on the WHO website and is available here:

10. ACKNOWLEDGEMENTS
We thank the donors of the original source material used to produce the standard and all of the participants in the collaborative study.

11. FURTHER INFORMATION
Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK
Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION
In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified
https://nibsc.org/documents/ifu/msds/MSDS-25-168.pdf

15. LIABILITY AND LOSS
In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.
Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

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16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.5g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached**: False

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards https://www.who.int/publications/m/item/annex2-trs932(revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.